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1 501 864

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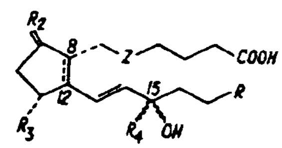
(54) PROSTAGLANDIN DERIVATIVES AND THEIR PREPARATION

(71) We, GIST-BROCADES N.V., a Dutch Body Corporate of Wateringseweg 1, Delft, Holland, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

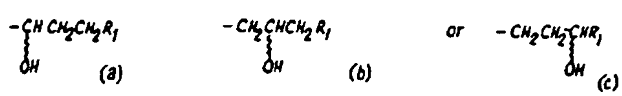
This invention relates to new therapeutically useful prostaglandin derivatives, to a new microbiological process for their preparation, to pharmaceutical compositions containing them and to their use in treating bronchospastic conditions.

The prostaglandin derivatives of the present invention are 18ξ-, 19ξ- and 20ξ-hydroxyprostaglandin derivatives of the general formula I,



I

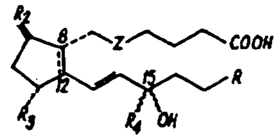
wherein the dotted line in the position 8—12 indicates the optional presence of a double bond, the wavy lines in position 15 indicate that the hydroxyl group and the group R_4 are either in the α- or β-configuration and Z represents a $—CH_2CH_2—$ or a *cis* —CH=CH— group, and wherein R represents one of the groups:



(wherein the wavy lines indicate that the hydroxyl groups are either in the α- or β-configuration and R_1 represents a hydrogen atom, a methyl or ethyl group), R_2 represents either an oxygen atom or a β- or α-hydrogen atom and an α- or β-hydroxyl group, R_3 represents a hydrogen atom or a hydroxyl group and R_4 represents a hydrogen atom or a methyl group, with the proviso that (i) when R_1, R_3 and R_4 each represents a hydrogen atom, R_2 represents an oxygen atom, a double bond is in the 8—12 position and the 15-hydroxyl group is in the α- or β-configuration, R does not represent the group (b), and (ii) when R_1, R_3 and R_4 each represents a hydrogen atom, R_2 represents an oxygen atom, the 15-hydroxyl group is in the α-configuration, Z represents a *cis* —CH=CH— group and the 8—12 position is saturated, R does not represent the group (a), and (iii) when there is a double bond in the 8—12 position, R_3 does not represent a hydroxyl group and (iv)

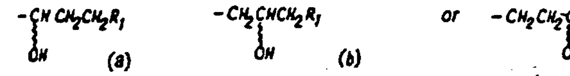
when there is a double bond in the 8—12 position, R_2 does not represent a β- or α-hydrogen and an α- or β-hydroxyl group; and the pharmaceutically acceptable salts and alkyl esters thereof.

The present invention provides also a process for the preparation of 18ξ-, 19ξ- and 20ξ-hydroxy-prostaglandin derivatives of the general formula IA



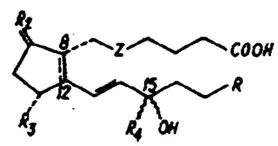
IA

wherein the dotted line in the position 8—12 indicates the optional presence of a double bond, the wavy lines in position 15 indicate that the hydroxyl group and the group R_4 are either in the α- or β-configuration and Z represents a $—CH_2CH_2—$ or a *cis* —CH=CH— group, and wherein R represents one of the groups:



(a) (b) or (c)

(wherein the wavy lines indicate that the hydroxyl groups are either in the α- or β-configuration and R_1 represents a hydrogen atom, a methyl or ethyl group), R_2 represents either an oxygen atom or a β- or α-hydrogen atom and an α- or β-hydroxyl group, R_3 represents a hydrogen atom or a hydroxyl group and R_4 represents a hydrogen atom or a methyl group, with the proviso that (1) when there is a double bond in the 8—12 position, R_3 does not represent a hydroxyl group and (2) when there is a double bond in the 8—12 position, R_2 does not represent a β- or α-hydrogen and an α- or β-hydroxyl group; which comprises subjecting a compound of the general formula II,



II

wherein the dotted line in the position 10—11 indicates the optional presence of a double bond in which case the 8—12 position is saturated and R_3 represents hydrogen, and the other symbols are as defined above, to the hydroxylating activity of (i) microorganisms (or enzymes thereof) of the Division of *Eumycota* or, (ii) when it is desired to prepare an 18- or 19-hydroxy prostaglandin derivative, microorganisms (or enzymes thereof) of the Family of *Streptomycetaceae* and, if desired, converting the resulting hydroxy-prostaglandin derivative of formula I into a pharmaceutically acceptable salt or alkyl ester thereof, with the proviso that when the microorganism is *Cunninghamella blakesleena* (ATCC 9245), the compound of formula II is not 15(S)-hydroxy-9-oxo-prosta-5(c), 10(t), 13(t)-trienoic acid (PGA_2).

The *Eumycota* used in this invention are of the Kingdom of Fungi, the Family of *Streptomycetaceae* used in the invention are of the Order Actinomycetales, Class Schizomycetes, Division Protophyta of the Kingdom of Plants.

The 18ξ-, 19ξ- and 20ξ-hydroxy-prostaglandin derivatives obtained can be converted into pharmaceutically acceptable salts and esters thereof, by reacting the corresponding compound in the form of a free acid with a suitable organic or inorganic base, e.g. an amine or hydroxy amine or an alkali metal hydroxide, or ester-forming derivative to form, for example, a C_1—C_4 alkyl ester.

Microbiological conversions of prostaglandins or of prostaglandin-type compounds have been described before, but these conversions usually relate to the reduction of oxo groups, mostly by bacteria or yeasts, for example the conversion of 9,15-dioxo-11-hydroxyprosta-8(12),13(t)-dienoic acid by *Flavobacterium* and *Pseudomonas* species into 9-oxo-11,15-dihydroxyprosta-8(12),13(t)-dienoic acid (M.Miyano *et al.*, Chem. Comm. (1971), 425).

U.K. Patent Specification No. 1,400,936 describes the fermentative reduction of the 10(11) double bond in PGA-type prostaglandins, sometimes accompanied by concomitant transformations, such as reduction of the 13(14) double bond or

oxidation of the 15-hydroxyl group to a 15-oxo group. In one particular case, *viz.* reduction of the 10(11) double bond in 9-oxo-15α-hydroxyprosta-5(c),10,13(t)-trienoic acid (PGA_2) with *Cunninghamella blakesleeana* (ATCC 9245), an 18-hydroxyl group is introduced.

The 19-hydroxyl derivatives of PGB_1 (9-oxo-15α-hydroxy-prosta-8(12),13(t)-dienoic acid) and PGB_2 (9-oxo-15α-hydroxyprosta-5,(c),8(12),13(t)-trienoic acid) are described by S. Bergstrom, Science 157, p. 382 ff (1967).

Prostaglandins are members of a new hormonal system with a remarkable range of biological and pharmaceutical properties. These compounds belong to a group of chemically related 20-carbon chain hydroxy fatty acids containing a five membered ring in the structure and different degrees of unsaturation, a number of which have been reported in the literature. For a review on prostaglandins and the definition of primary prostaglandins, see, for example, S. Bergstrom, Recent Progress in Hormone Research, *22*, pp. 153—175 (1966) and Science, *157*, p. 382 ff (1967) by the same author.

Prostaglandins are widely distributed in mammalian tissues and have been isolated from natural sources in very small amounts. In addition, a number of the naturally occurring prostaglandins have been prepared by chemical synthesis; note, for example, J. Am. Chem. Soc., *91*, p. 5675 ff (1969); J. Am. Chem. Soc., *92*, p. 2586 ff (1970) and J. Am. Chem. Soc., *93*, pages 1489—1493 (1971) and references cited therein; W. P. Schneider *et al.*, J. Am. Chem. Soc., *90*, p. 5895 ff (1968); U. Axen et al., Chem. Commun., p. 303 ff (1969) and W. P. Schneider, Chem. Commun. p. 304 ff (1969).

Because of the remarkable range of biological and pharmacological properties exhibited by this family of compounds, a great deal of interest has focused upon such compounds, and the preparation of analogs of such compounds.

The 18ξ-, 19ξ- and 20ξ-hydroxy-prostaglandin derivatives of general formula I are potent agents in the treatment of bronchial asthma and other bronchospastic conditions. They have considerable relaxant activity on respiratory smooth muscle, whereas they were found, in general, to be devoid of appreciable activity on the intestinal and uterine smooth muscle, as well as of appreciable irritant activity at the site of application.

The utility of various prostaglandins and prostaglandin-derivatives presently in clinical use is limited due to the occurrence of undesirable side-effects, such as diarrhoea, abdominal cramps and/or irritation at the site of application.

The selective activity of the hydroxy-prostaglandin derivatives of the present invention was established by a multiparameter guinea-pig test. In this test guinea-pigs weighing 600—900 g are anaesthetized with sodium pentobarbitone (45 mg/kg, i.p.). Supplementary doses of sodium pentobarbitone (3—6 mg i.v.) are administered when required (i.e. when spontaneous respiration appears). The jugular vein is cannulated for the administration of drugs. The guinea-pig is artifically respired with N_2O/O_2 (7/3), using a Keuskamp respirator.

Then the following functions are measured:

a. Blood pressure.

The common carotid artery is cannulated and the blood pressure measured with a pressure transducer.

b. Bronchial resistance and tracheal segment pressure.

A cannula is inserted into the trachea as close as possible to the thorax. The guinea-pig is artifically ventilated at 55 strokes/min. The pressure changes, assumed to be due to changes caused by the bronchioles, are measured by a pressure transducer attached to a side arm of the cannula. The trachea is occluded at its lower end with a blind-ended cannula, while a cannula is further introduced into the trachea as close as possible to the larynx. The system is completely filled with saline, and connected to a very sensitive pressure transducer. Changes in the pressure measured (cm H_2O) are assumed to reflect changes in the tone of the smooth muscle of the trachea. The trachea segment cannula is inserted with extreme caution so as to avoid disruption of the nerve or blood supply to the segment.

c. Measurement of intestinal motility.

A balloon, containing distilled water and connected to a pressure transducer, is inserted in the duodenum of the guinea-pig. Care is taken on ligaturing the

cannula to avoid stricture of the duodenum. The balloon is at a pressure of 10—20 mm Hg.

d. Measurement of uterine motility.

A polyethylene cannula is inserted into the uterus via the vagina to a depth of 2.5 cm. This is then tied off with a ligature around the cervix. The cannula is connected to a pressure transducer, the whole system being filled with liquid paraffin at a pressure of 10—20 mm Hg.

The present hydroxy-prostaglandin derivatives compare favourably in this multiparameter test with well-known prostaglandins, such as $PGF_{2\alpha}$ and PGE_1, as is demonstrated for some compounds of this invention by Table 1.

TABLE 1

Guinea-pig multiparameter test.

COMPOUND	DOSE in μg	TRACHEAL segment pressure	BRONCHIAL resistance.	INTESTINAL contractions	UTERINE contractions.
$PGF_{2\alpha}$	20	+	+	+	++
PGE_1	5	–	0	+	0
9-oxo-15α,18ξ-dihydroxy-prost-13(t)-enoic acid	100	–	0	0	0
9-oxo-15α,19ξ-dihydroxy-prost-13(t)-enoic acid	100	–	0	0	0
9β,15α,18ξ-trihydroxy-prost-13(t)-enoic acid	500	–	0	0	0
9β,15α,19ξ-trihydroxy-prost-13(t)-enoic acid	500	–	0	0	0
9β,15α,20-trihydroxy-prost-13(t)-enoic acid	500	–	0	0	0

The activity of the 18ξ-, 19ξ- and 20ξ-hydroxy-prostaglandin derivatives on the respiratory tract musculature was further confirmed by determination of their ability to antagonise histamine-induced bronchoconstriction. This test is a modification of the guinea-pig multiparameter test, cannulations being carried out only for recording blood pressure, tracheal segment pressure and bronchial resistance.

Histamine was injected i.v. in a dose of 4 μg (as base) at regular intervals throughout the experiment. If extra doses of sodium pentobarbitone had to be administered during the course of the experiment to suppress voluntary respiration, the interval to the next dose of histamine was lengthened.

Test compounds were injected i.v. one minute before histamine in volumes less than 0.5 ml. The substances were washed in with 0.3 ml sterile saline. The lungs were artificially over-ventilated one minute prior to injection of the test compounds.

The ability of the compounds to counteract histamine-induced bronchoconstriction and the increase in tracheal segment pressure was determined using two dose levels — a low one and a high one.

Some of the results obtained with compounds according to this invention, using PGE_1 as the reference compound, are shown in Table 2.

TABLE 2

Antagonism of Histamine-induced Broncho-constriction (guinea-pig).

COMPOUND	DOSE in μg	% INHIBITION ($\pm$ S.D.*)
PGE_1	0.1	27.6 ($\pm$ 10.5)
	1.0	53.2 ($\pm$ 14.8)
	5.0	about 88
9-oxo-15α,18ξ-dihydroxy-prost-13(t)-enoic acid	1.0	25.9 ($\pm$ 6)
	100	about 80
9-oxo-15α,19ξ-dihydroxy-prost-13(t)-enoic acid	1	23.9 ($\pm$ 13.4)
	100	88.1 ($\pm$ 5.0)
9β,15α,18ξ-trihydroxy-prost-13(t)-enoic acid	100	28.1 ($\pm$ 15.2)
9β,15α,19ξ-trihydroxy-prost-13(t)-enoic acid	100	62.5 ($\pm$ 6.6)
9β,15α,20-trihydroxy-prost-13(t)-enoic acid	100	52.9 ($\pm$ 17.9)
9α,15β,19ξ-trihydroxy-prost-13(t)-enoic acid	500	about 60
9-oxo-15β,19ξ-dihydroxy-prost-13(t)-enoic acid	500	about 85
9-oxo-15α,19ξ-dihydroxy-prosta-5(c),13(t)-dienoic acid	1	50.3 ($\pm$ 4.3)
9-oxo-15α,20-dihydroxy-prosta-5(c),13(t)-dienoic acid	1	60.7 ($\pm$ 14.6)
9-oxo-11α,15α,18ξ-trihydroxy-prost-13(t)-enoic acid	1	about 70
9-oxo-11α,15α,19ξ-trihydroxy prost-13(t)-enoic acid	1	about 60
9-oxo-11α,15α,18ξ-trihydroxy-prosta-5(c),13(t)-dienoic acid	1	about 35
9-oxo-11α,15α,19ξ-trihydroxy-prosta-5(c),13(t)-dienoic acid	1	about 70

* S.D. is standard deviation i.e. the range within which changes are insignificant.

The irritation at the site of application which is shown by various prostaglandins and prostaglandin derivatives can result in phlebitis at the site of injection or in persistant coughing if (as in the case for example with PGE_1 and PGE_2) an aerosol is employed.

This effect can be studied using the Draize scoring method for determining irritation following topical application in the rabbit eye. PGE_1 was used as the reference compound; 1 μg/eye was the threshold irritant dose with this compound;

5 μg was definitely irritant. Doses of the present hydroxy-prostaglandin derivatives which were equieffective or more effective than PGE_1 against histamine induced bronchoconstriction, proved not to irritate the rabbit eye by topical application. The results for some compounds of the invention are given in Table 3.

TABLE 3

COMPOUND	DOSE in μg	IRRITATION
PGE_1	5	YES
9-oxo-15α,19ξ-dihydroxy-prost-13(t)-enoic acid	100	NO
9-oxo-15α,20-dihydroxy-prosta-5(c),13(t)-dienoic acid	100	NO
9-oxo-11α,15α,19ξ-trihydroxy-prost-13(t)-enoic acid	25	NO
9-oxo-11α,15α,19ξ-trihydroxy-prosta-5(c),13(t)-dienoic acid	25	NO

From the results obtained it may be concluded in view of the explanations given above, that the 18ξ-, 19ξ- and 20ξ-prostaglandin derivatives of the present invention are particularly useful for the treatment of bronchial asthma and other bronchospastic conditions. Their advantages over various of the presently available prostaglandin derivatives, are that they either have greater specificity (i.e. less or absent activity on the intestines) or are less irritant at the site of application, or both.

Specific new prostaglandin compounds of this invention are the 18ξ-, 19ξ- and 20ξ-hydroxy derivatives of the following prostaglandins and prostaglandin-type compounds:
9-oxo-11α,15α-dihydroxy-prost-13(t)-enoic acid;
9-oxo-11α,15α-dihydroxy-prosta-5(c),13(t)-dienoic acid;
9α,11α,15α-trihydroxy-prosta-5(c),13(t)-dienoic acid;
9-oxo-15α-hydroxy-prost-13(t)-enoic acid;
9α,15α- and 9β,15α-dihydroxy-prost-13(t)enoic acid;
9-oxo-15β-hydroxy-prost-13(t)-enoic acid;
9α,15β- and 9β,15β-dihydroxy-prost-13(t)-enoic acid;
9α,15α-dihydroxy-15β-methyl-prost-13(t)-enoic acid;
9α,15α-dihydroxy-20-ethyl-prost-13(t)-enoic acid;
9-oxo-15α-hydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid;
9-oxo-15β-hydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid;
9α-15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid;
9α,15β-dihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid;

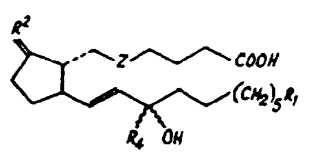
Specific prostaglandins and prostaglandin-type compounds of the general formula II which can be microbiologically hydroxylated according to the process of this invention include:
9-oxo-15α-hydroxy-prosta-5(c),10,13(t)-trienoic acid;
9-oxo-15α-hydroxy-prosta-5(c),8(12),13(t)-trienoic acid;
9-oxo-11α,15α-dihydroxy-prost-13(t)-enoic acid;
9-oxo-11α,15α-dihydroxy-prosta-5(c),13(t)-dienoic acid;
9α,11α,15α- and 9β,11α,15α-trihydroxy-prost-13(t)-enoic acid;
9α,11α,15α- and 9β,11α,15α-trihydroxy-prosta-5(c),13(t)-dienoic acid;
dl-9α,15α-,9β,15α-, 9α,15β- and 9β,15β-dihydroxy-prost-13(t)-enoic acid;
dl-9-oxo-15α- and 15β-hydroxy-prost-13(t)-enoic acid;
dl-9α,15α,9β,15α-, 9α,15β- and 9β,15β-dihydroxy-prosta-5(c),13(t)-dienoic acid;
dl-9-oxo-15α- and 15β-hydroxyprosta-5(c),13(t)-dienoic acid;
dl-9α,15α-dihydroxy-15β-methyl-prost-13(t)-enoic acid;
dl-9α,15β-dihydroxy-15α-methyl-prost-13(t)-enoic acid;
dl-9β,15α-dihydroxy-15β-methyl-prost-13(t)-enoic acid;
dl-9β,15β-dihydroxy-15α-methyl-prost-13(t)-enoic acid;
dl-9-oxo-15α-hydroxy-15β-methyl-prost-13(t)-enoic acid;

dl-9-oxo-15β-hydroxy-15α-methyl-prost-13(t)-enoic acid;
dl-9α,15α-dihydroxy-15β-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9α,15β-dihydroxy-15α-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9β,15α-dihydroxy-15β-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9β,15β-dihydroxy-15α-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9-oxo-15α-hydroxy-15β-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9-oxo-15β-hydroxy-15α-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9α,15α,9β,15α-, 9α,15β-, and 9β,15β-dihydroxy-20-methyl-prost-13(t)-enoic acid;
dl-9-oxo-15α- and 15β-hydroxy-20-methyl-prost-13(t)-enoic acid;
dl-9α,15α, 9β,15α-, 9α,15β- and 9β,15β-dihydroxy-20-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9-oxo-15α- and 15β-hydroxy-20-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9α,15α, 9β,15α-, 9α,15β- and 9β,15β-dihydroxy-20-ethyl-prost-13(t)-enoic acid;
dl-9-oxo-15α- and 15β-hydroxy-20-ethyl-prost-13(t)-enoic acid;
dl-9α,15α-, 9β,15α-, 9α,15β- and 9β,15β-dihydroxy-20-ethyl-prosta-5(c),13(t)-dienoic acid;
dl-9-oxo-15α- and 15β-hydroxy-20-ethyl-prosta-5(c),13(t)-dienoic acid;
dl-9α,15α-dihydroxy-15β-methyl-20-methyl-prost-13(t)-enoic acid;
dl-9α,15β-dihydroxy-15α-methyl-20-methyl-prost-13(t)-enoic acid;
dl-9β,15α-dihydroxy-15β-methyl-20-methyl-prost-13(t)-enoic acid;
dl-9β,15β-dihydroxy-15α-methyl-20-methyl-prost-13(t)-enoic acid;
dl-9-oxo-15α-hydroxy-15β-methyl-20-methyl-prost-13(t)-enoic acid;
dl-9-oxo-15β-hydroxy-15α-methyl-20-methyl-prost-13(t)-enoic acid;
dl-9α,15α-dihydroxy-15β-methyl-20-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9α,15β-dihydroxy-15α-methyl-20-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9β,15α-dihydroxy-15β-methyl-20-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9β,15β-dihydroxy-15α-methyl-20-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9-oxo-15α-hydroxy-15β-methyl-20-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9-oxo-15β-hydroxy-15α-methyl-20-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid;
dl-9α,15β-dihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid;
dl-9β,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid;
dl-9β,15β-dihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid;
dl-9-oxo-15α-hydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid;
dl-9-oxo-15β-hydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid;
dl-9α,15α-dihydroxy-15β-methyl-20-ethyl-prosta-5(c),13(t)-dienoic acid;
dl-9α,15β-dihydroxy-15α-methyl-20-ethyl-prosta-5(c),13(t)-dienoic acid;
dl-9β,15α-dihydroxy-15β-methyl-20-ethyl-prosta-5(c),13(t)-dienoic acid;
dl-9β,15β-dihydroxy-15α-methyl-20-ethyl-prosta-5(c),13(t)-dienoic acid;
dl-9-oxo-15α-hydroxy-15β-methyl-20-ethyl-prosta-5(c),13(t)-dienoic acid;
dl-9-oxo-15β-hydroxy-15α-methyl-20-ethyl-prosta-5(c),13(t)-dienoic acid;

Some of the starting materials useful in preparing the novel 18ξ-, 19ξ- and 20ξ-hydroxy-prostaglandin derivatives of general formula I are known substances, such as:

9-oxo-15α-hydroxy-prosta-5(c),10,13(t)-trienoic acid (PGA_2);
9-oxo-15α-hydroxy-prosta-5(c),8(12),13(t)-trienoic acid (PGB_2);
9-oxo-11α,15α-dihydroxy-prost-13(t)-enoic acid (PGE_1);
9-oxo-11α,15α-dihydroxy-prosta-5(c),13(t)-dienoic acid (PGE_2);
9α,11α,15α-trihydroxy-prost-13(t)-enoic acid ($PGF_{1\alpha}$);
9β,11α,15α-trihydroxy-prost-13(t)-enoic acid ($PGF_{1\beta}$);
9α,11α,15α-trihydroxy-prosta-5(c),13(t)-dienoic acid ($PGF_{2\alpha}$);
9β,11α,15α-trihydroxy-prosta-5(c),13(t)-dienoic acid ($PGF_{2\beta}$).

Other starting materials in the process of this invention with the general formula III,



III

wherein Z, R_1, R_2 and R_4 are as defined above, can be prepared according to the abbreviated schematic reaction sequence shown in Figure 1, wherein each of the symbols A, B, IV and V to IX represents compounds whose structures are shown in

Figure 2, wherein Z and R_1 are as defined above, and the waved line in formula IV indicates a mixture of the α- and β-isomer.

The compounds of formula III (the free acids) are obtained by alkaline hydrolysis of the corresponding methyl esters of the formulas V, VI, VIII and IX shown in Figure 2.

The compounds of formula A, wherein R_1 is as defined above, which are starting material in the reaction sequence shown in Figure 2, are conveniently prepared according to the schematic overall reaction sequence shown in Figure 3.

Fig. 1

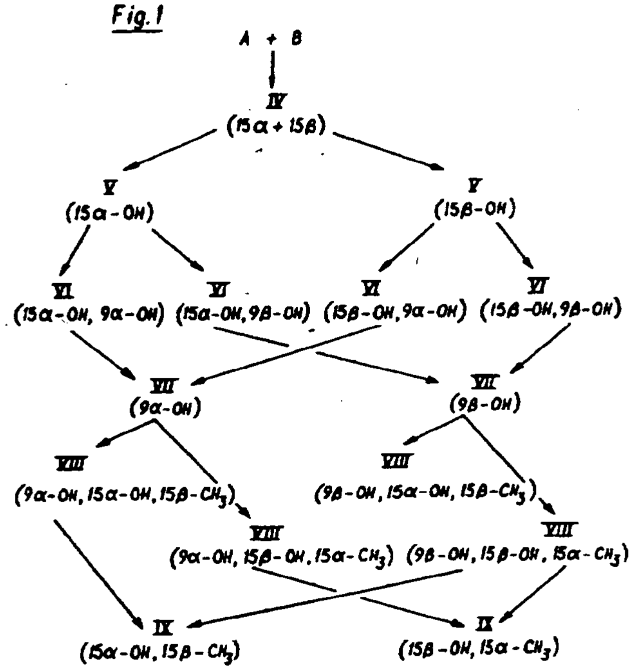


Fig. 2

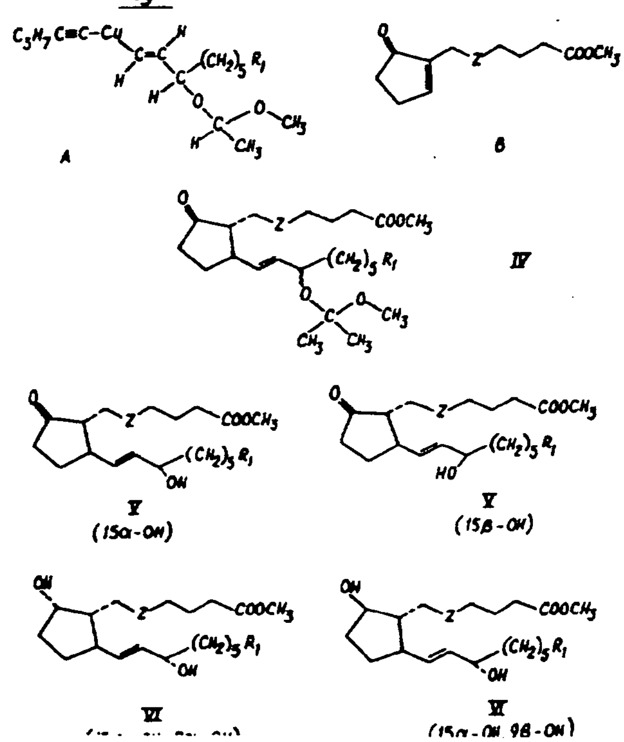


Fig. 2 - cont'd

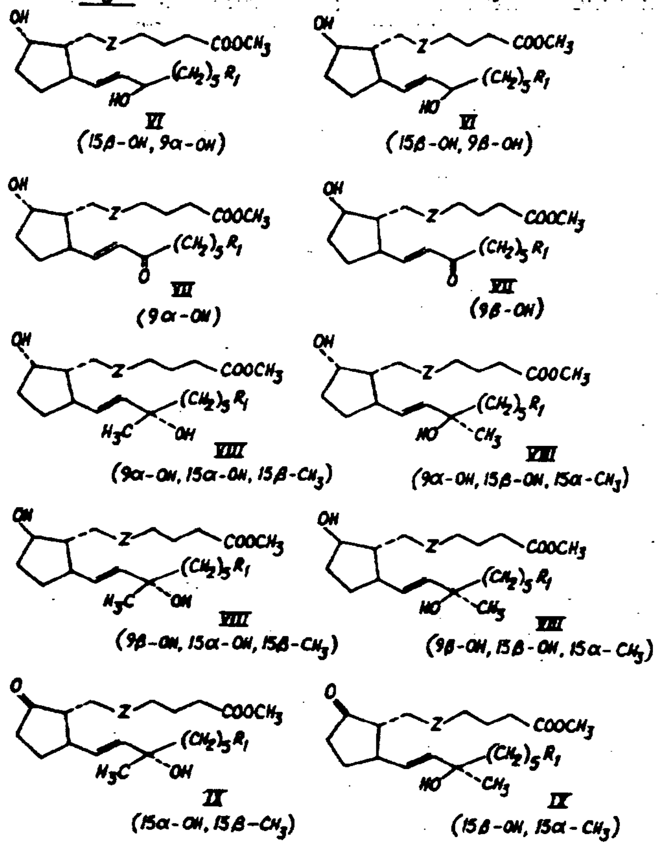
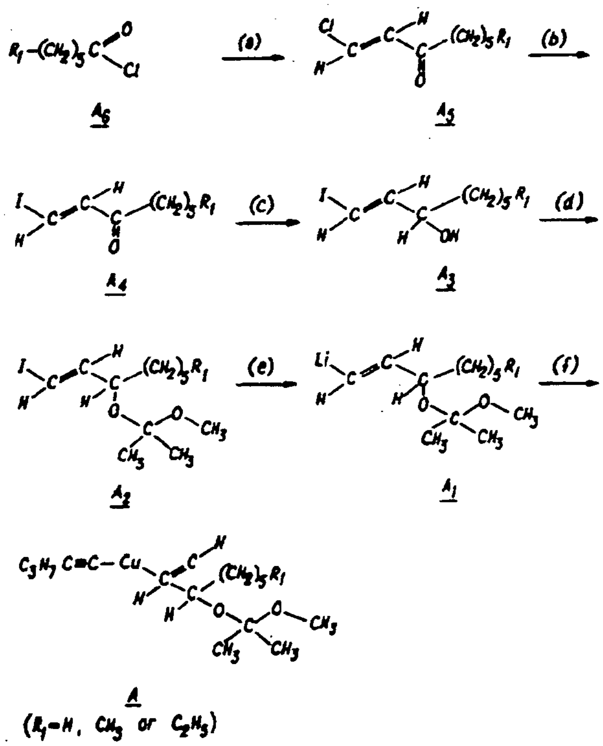


Fig. 3



The compounds of formula A can be prepared as follows:

Step (a) can be carried out by treating the compounds of formula A_6 with acetylene in the presence of aluminium chloride at 0°C to give the compounds of formula A_5. The reaction is usually complete within four hours.

Step (b) can be carried out by treating the compounds of formula A_5 with sodium iodide under anhydrous conditions and is typically conducted in acetone under reflux until the reaction is complete, usually from three to twelve hours, to obtain the compounds of formula A_4.

Step (c) can be carried out by treating compounds of formula A_4 with sodium bis(2-methoxy ethoxy)aluminium hydride and subsequently with an acid, e.g., sulfuric acid, at 0°C to produce the compounds of formula A_3.

Step (d) is conveniently effected by treating the compounds of formula A_3 with isopropenyl methyl ether in the presence of an acid catalyst e.g., dichloroacetic acid or phosphorous oxychloride, at 0°C. The compound of formula A_2 wherein R_1 is a hydrogen atom, is also disclosed by Kluge *et al.*, J. Am. Chem. Soc., 94,7827 (1972).

Step (e) can be carried out by treating compounds of formula A_2 with t-butyl lithium at −78°C to give the compounds of formula A_1.

The last step of the above preparation, step (f), is conveniently effected by adding a solution of the compounds of formula A_1 to a solution of copper pentyne and hexamethylphosphoroustriamide to give the compounds of formula A. The reaction may be carried out at −78°C and is usually complete within one hour.

The compounds of formula IV are conveniently prepared by adding to the freshly prepared compounds of formula A, the preparation of which is described above, a compound of formula B, described by Bagli *et al.* in Tetrahedron Letters, 465—470 (1966). The reaction is conveniently carried out at −78°C and gives a mixture of two isomers of formula IV.

The compounds of formula V are conveniently prepared by removing the ether protecting group by treating the above obtained mixture of compounds of formula IV with acetic acid at room temperature. The resulting mixture of the compounds of formula V can be separated into its isomers (15α—OH and 15β—OH) by means of chromatography on silica gel using ethyl acetate/hexane of increasing polarity as solvent.

The resulting compounds of formula V (15α—OH) can be converted to a mixture of the isomers of the compounds of formula VI (15α—OH, 9α—OH and 15α—OH, 9β—OH) by treatment with sodium borohydride at 0°C. The reaction is usually complete within about 45 minutes. The mixture of isomers can then be chromatographed on silica gel using ethyl acetate/hexane of increasing polarity as the solvent to give the compounds of formula VI (15α—OH, 9α—OH and 15α—OH, 9β—OH). In a similar manner, the compounds of formula V (15β—OH) can be converted into the individual isomers, the compounds of formula VI (15β—OH, 9α—OH and 15β—OH, 9β—OH).

The resulting compounds of formula VI (15α—OH, 9α—OH or 15β—OH, 9α—OH) can be treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone for 36 hours at room temperature in a benzene solution to give the compounds of formula VII (9α—OH). Similarly, substituting the compounds of formula VI (15α—OH, 9β—OH or 15β—OH, 9β—OH) for the compounds of formula VI (15α—oh, 9α—OH) gives the compounds of formula VII (9β—OH).

Treatment of the compounds of formula VII (9α—OH) with methylmagnesium bromide in tetrahydrofuran at −30°C for 45 minutes gives a mixture of compounds of formula VIII (9α—OH, 15α—OH, 15β—CH_3 and 9α—OH, 15β—OH, 15α—CH_3), which can be separated into individual isomers by chromatography on silica gel using ethyl acetate/hexane of increasing polarity as solvent. Substituting the compounds of formula VII (9β—OH) for VII (9α—OH) in the above reaction gives the compounds of formula VIII (9β—OH, 15α—OH, 15β—CH_3 and 9β—OH, 15β—OH, 15α—CH_3).

The resulting compounds of formula VIII (9α—OH, 15α—OH, 15β—CH_3) can be treated with a suspension of Celite (diatomaceous earth) and chromium trioxide in anhydrous methylene chloride under nitrogen in the presence of pyridine for about one hour to give the compounds of formula IX (15α—OH, 15β—CH_3). "Celite" is a Registered Trade Mark. Compound IX can also be obtained by substituting the compounds of formula VIII (9β—OH, 15α—OH, 15β—CH_3) for the compounds of formula VIII (9α—OH, 15α—OH, 15β—CH_3). Substituting the compounds of formula VIII (9α—OH, 15β—OH, 15α—CH_3 or 9β—OH, 15β—OH, 15α—CH_3) for the compounds of formula VIII (9α—OH,

15α—OH, 15β—CH_3) gives the compounds of formula IX (15β—OH, 15α—CH_3).

The compounds of formulae V, VI, VIII and IX can be converted to their corresponding free acids by treatment with base, e.g., potassium hydroxide at room temperature for about 2 hours, to give the compounds of formula III.

The preparation of certain intermediates used in the production of the hydroxy prostaglandins of the invention will now be described in the following Preparations.

PREPARATION 1.

This preparation illustrates methods for preparing *dl*-1-pentynyl-1-[3-(2,2-methoxypropoxy)-*trans*-1-decenyl]cuprate. (A, R_1=C_2H_5)

(a) A solution of 200 ml of octanoyl chloride (A_6, R_1=C_2H_5) in 750 ml of Carbon tetrachloride is cooled on an ice bath and treated with 214 g of aluminium chloride in three portions over a 1 hour period while acetylene is bubbled through the solution. The ice bath is removed and the reaction mixture stirred at room temperature for 3 hours with additional acetylene being added. At the end of this period, the reaction mixture is poured into 4 kg of ice. The organic layer is separated and the aqueous layer extracted twice with 500 ml of chloroform. The combined organic extracts are washed once with 500 ml of water, dried over anhydrous sodium sulfate and concentrated *in vacuo*. Distillation of the residue gives 142 g of 1-chloro-dec-*trans*-1-en-3-one, (A_5, R_1=C_2H_5).

(b) A solution of 142 g of the product of (a), 140 g of sodium iodide and 500 ml of acetone is refluxed under nitrogen for 4 hours. The acetone is then removed under reduced pressure and the residue dissolved in 500 ml of water. The mixture is extracted twice with 400 ml of ether, the ether extracts combined and washed with 5% aqueous sodium thiosulfate, then with saturated sodium chloride and finally dried over anhydrous sodium sulfate. The ether is removed *in vacuo* to give 1-iododec-trans-1-en-3-one (A_4, R_1=C_2H_5).

(c) The crude product of (b) is dissolved in 750 ml of benzene, cooled on an ice bath under nitrogen and then treated with 140 ml of 65% sodium bis(2-methoxyethoxy)aluminium hydride over a one hour period. After stirring the mixture for an additional 30 minutes at 0°C, 38 ml of concentrated sulfuric acid in 120 ml of water are added. The reaction mixture is then filtered and the filtrate washed twice with 500 ml of saturated sodium chloride. The benzene is removed *in vacuo* and the residue distilled to give 159 g of *dl*-1-iodo-3-hydroxy-*trans*-1-decene, (A_3, R_1=C_2H_5).

(d) A solution of 5.64 g of the product of (c) in 8 ml of isopropenyl methyl ether is cooled to 0°C and treated with 5 drops of dichloroacetic acid. The ice bath is then removed and the reaction allowed to proceed at room temperature for 1 hour. Five drops of triethyl amine are then added and the excess isopropenyl methyl ether removed *in vacuo* to yield 7.5 g of *dl*-1-iodo-3-(1-methoxy-1-methylethoxy)-*trans*-1-decene, (A_2, R_1=C_2H_5).

(e) 7.5 g of the product of (d) are dissolved in 30 ml of diethyl ether and cooled to −78°C under nitrogen. 32 ml of 1.25 N t-butyl lithium are then added over 30 minutes while maintaining the reaction temperature near −70°C. The reaction mixture is stirred at −78°C for 45 minutes to give *dl*-1-lithio-3-(1-methoxy-1-methylethoxy)-*trans*-1-decene, (A_1, R_1=C_2H_5).

(f) The resulting solution of lithium reagent is added to a solution of 2.60 g of copper pentyne and 7.9 ml of hexamethylphosphoroustriamide in 100 ml of diethyl ether, also at −78°C. This mixture is allowed to stir at −78°C for 15 minutes to yield *dl*-1-pentynyl-1-[3-(1-methoxy-1-methylethoxy)-*trans*-1-decenyl]-cuprate, (A, R_1=C_2H_5).

Similarly, by substituting hexanoyl chloride and heptanoyl chloride for octanoyl chloride in step (a), and by following the procedure as described in steps (a) to (f) above, *dl*-1-pentynyl-1-[3-(1-methoxy-1-methylethoxy)-*trans*-1-octenyl]-cuprate, (A, R_1=H), and *dl*-1-pentynyl-1-[3-(1-methoxy-1-methylethoxy)-*trans*-1-nonenyl] cuprate, (A, R_1=CH_3), respectively are prepared.

PREPARATION 2.

This preparation illustrates methods of preparing methyl *dl*-9-oxo-15α(β)-(1-methoxy-1-methylethoxy)-20-ethyl-prost-13(t)-enoate (IV, R_1=C_2H_5, Z=CH_2CH_2).

In this preparation a solution of 4.0 g of 2-(6-carbomethoxyhexyl)-cyclopent-2-en-1-one (B, Z=CH_2CH_2) in 10 ml of diethyl ether is added to a freshly prepared solution of *dl*-1-pentynyl-1-[3-(1-methoxy-1-methylethoxy)-*trans*-1-decenyl]cuprate (A, R_1=C_2H_5), prepared as described in Preparation 1. The reaction mixture is

stirred at $-78°C$ for 1 hour and then poured into 250 ml of ice water. The organic layer is separated and the aqueous layer extracted twice with 100 ml of diethyl ether. The combined organic layers are dried over anhydrous sodium sulfate and concentrated *in vacuo* to yield a mixture of methyl *dl*-9-oxo-15α- and 15β-(1-methoxy-1-methylethoxy)-20-ethyl-prost-13(t)-enoate.

Similarly, by following the same procedure but replacing *dl*-1-pentynyl-1-[3-(1-methoxy-1-methylethoxy)-*trans*-1-decenyl]cuprate by *dl*-1-pentynyl-1 - [3 - (1-methoxy-1-methylethoxy)-*trans*-1-octenyl]cuprate or by *dl*-1-pentynyl-1 - [3 - (1-methoxy-1-methylethoxy)-*trans*-1-nonenyl)cuprate, the following compounds of formula IV are prepared:

methyl *dl*-9-oxo-15α- and 15β-(1-methoxy-1-methylethoxy)-prost-13(t)-enoate
methyl *dl*-9-oxo-15α- and 15β-(1-methoxy-1-methylethoxy)-20-methylprost-13(t)-enoate.

In a similar manner, by substituting 2-(6-carbomethoxy-2-*cis*-hexenyl)-cyclopent-2-en-1-one for 2-(6-carbomethoxyhexyl)-cyclopent-2-en-1-one, a mixture of methyl *dl*-9-oxo-15α- and 15β-(1-methoxy-1-methylethoxy)-20-ethyl-prosta-5(c), 13(t)-dienoate is obtained.

Similarly, by substituting *dl*-1-pentynyl-1-[3-(1-methoxy-1-methylethoxy)-*trans*-1-octenyl]cuprate or *dl*-1-pentynyl-1-[3-(1-methoxy-1-methylethoxy)-*trans*-1-nonenyl]cuprate and by substituting 2-(6-carbomethoxy-2-*cis*-hexenyl)-cyclopent-2-en-1-one for 2-(6-carbomethoxyhexyl)-cyclopent-2-en-1-one and following the same procedure as described above the following compounds are obtained:
methyl *dl*-9-oxo-15α- and 15β-(2,2-methoxypropoxy)-prosta-5(c), 13(t)-dienoate,
methyl *dl*-9-oxo-15α- and 15β-(1-methoxy-1-methylethoxy)-20-methylprosta-5(c), 13(t)-dienoate.

PREPARATION 3.

This preparation illustrates methods for removing the ether protecting group (1-methoxy-1-methylethoxy) from the products of formula IV of Preparation 2. In this preparation, the mixture of methyl *dl*-9-oxo-15α-(1-methoxy-1-methylethoxy)-20-ethyl-prost-13(t)-enoate and methyl *dl*-9-oxo-15β-(1-methoxy-1-methylethoxy)-20-ethyl-prost-13(t)-enoate (IV, $R_1=C_2H_5$, $Z=CH_2CH_2$) obtained in Preparation 2 is dissolved in 50 ml of water, 50 ml of methanol and 20 ml of acetic acid and stirred at room temperature for 1 hour. The reaction mixture is diluted with 100 ml of water and extracted three times with 200 ml of diethyl ether. The etheral layers are washed with 500 ml of saturated sodium chloride, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The resulting residue is chromatographed on 400 g of silica gel and eluted with 20% ethyl acetate — hexane (v/v), to give 2.509 g of methyl *dl*-9-oxo-15β-hydroxy-20-ethyl-prost-13(t)-enoate (V, 15β—OH, $R_1=C_2H_5$, $Z=CH_2CH_2$). Further elution with 25% ethyl acetate — hexane (v/v) gives 2.7 g of methyl *dl*-9-oxo-15α-hydroxy-20-ethyl-prost-13(t)-enoate (V, 15α—OH, $R_1=C_2H_5$, $Z=CH_2CH_2$).

Similarly, by following the same procedure as above, the ether protecting groups are removed from the other ether protected products prepared in Preparation 2 to give the following compounds of formula V which can be separated into their optically pure isomers by thin-layer preparative chromatography as described above:

methyl *dl*-9-oxo-15α- and 15β-hydroxy-prost-13(t)-enoate;
methyl *dl*-9-oxo-15α- and 15β-hydroxy-20-methyl-prost-13(t)-enoate;
methyl *dl*-9-oxo-15α- and 15β-hydroxy-20-ethyl-prosta-5(c),13(t)-dienoate;
methyl *dl*-9-oxo-15α- and 15β-hydroxy-prosta-5(c),13(t)-dienoate;
methyl *dl*-9-oxo-15α- and 15β-hydroxy-20-methyl-prosta-5(c),13(t)-dienoate.

PREPARATION 4.

This preparation illustrates methods for preparing methyl *dl*-9α,15α-dihydroxy - 20 - ethyl - prost - 13(t) - enoate (VI, 15α—OH, 9α—OH, $R_1=C_2H_5$, $Z=CH_2CH_2$), and methyl *dl*-9β,15α-dihydroxy-20-ethyl-prost-13(t)-enoate (VI, 15α—OH, 9β—OH, $R_1=C_2H_5$, $Z=CH_2CH_2$).

In this preparation, a solution of 1.7 g of methyl *dl*-9-oxo-15α-hydroxy-20-ethyl-prost-13(t)-enoate, prepared as described in Preparation 3, in 100 ml of ethanol is cooled on an ice bath and treated with 0.50 g of sodium borohydride. After 45 minutes at 0°C the reaction is quenched by addition of 1 ml of acetic acid. The reaction mixture is then diluted with 100 ml of water and extracted three

times with 200 ml of ethyl acetate. The combined ethyl acetate extracts are washed with saturated aqueous sodium chloride, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue is then chromatographed on 300 g of silica gel. Elution with 25% ethyl acetate/hexane (v/v) gives 425 mg of methyl *dl*-9α,15α-dihydroxy-20-ethyl-prost-13(t)-enoate. Further elution with 35% ethyl acetate/hexane (v/v) gives 1.12 g of methyl *dl*-9β,15α-dihydroxy-20-ethyl-prost-13(t)-enoate.

In similar manner, by substituting the other methyl ester 9-oxo-compounds prepared in Preparation 3, i.e.,

methyl *dl*-9-oxo-15β-hydroxy-20-ethyl-prost-13(t)-enoate;
methyl *dl*-9-oxo-15α-hydroxy-prost-13(t)-enoate;
methyl *dl*-9-oxo-15β-hydroxy-prost-13(t)-enoate;
methyl *dl*-9-oxo-15α-hydroxy-20-methyl-prost-13(t)-enoate;
methyl *dl*-9-oxo-15β-hydroxy-20-methyl-prost-13(t)-enoate;
methyl *dl*-9-oxo-15α-hydroxy-20-ethyl-prosta-5(c),13(t)-dienoate;
methyl *dl*-9-oxo-15β-hydroxy-20-ethyl-prosta-5(c),13(t)-dienoate;
methyl *dl*-9-oxo-15α-hydroxy-prosta-5(c),13(t)-dienoate;
methyl *dl*-9-oxo-15β-hydroxy-prosta-5(c),13(t)-dienoate;
methyl *dl*-9-oxo-15α-hydroxy-20-methyl-prosta-5(c),13(t)-dienoate; and
methyl *dl*-9-oxo-15β-hydroxy-20-methyl-prosta-5(c),13(t)-dienoate, for
methyl *dl*-9-oxo-15α-hydroxy-20-ethyl-prost-13(t)-enoate yields the following compounds of formula VI are prepared which are then separated into the optically pure isomers by thin-layer preparative chromatography,

methyl *dl*-9α,15β- and 9β,15β-dihydroxy-20-ethyl-prost-13(t)-enoate,
methyl *dl*-9α,15α- and 9β,15α-dihydroxy-prost-13(t)-enoate,
methyl *dl*-9α,15β- and 9β,15β-dihydroxy-prost-13(t)-enoate,
methyl *dl*-9α,15α- and 9β,15α-dihydroxy-20-methyl-prost-13(t)-enoate,
methyl *dl*-9α,15β- and 9β,15β-dihydroxy-20-methyl-prost-13(t)-enoate,
methyl *dl*-9α,15α- and 9β,15α-dihydroxy-20-ethyl-prosta-5(c),13(t)dienoate,
methyl *dl*-9α,15β- and 9β,15β-dihydroxy-20-ethyl-prosta-5(c),13(t)-dienoate
methyl *dl*-9α,15α- and 9β,15α-dihydroxy-prosta-5(c),13(t)-dienoate,
methyl *dl*-9α,15β- and 9β,15β-dihydroxy-prosta-5(c),13(t)-dienoate,
methyl *dl*-9α,15α- and 9β,15α-dihydroxy-20-methyl-prosta-5(c),13(t)-dienoate,
methyl *dl*-9α,15β- and 9β,15β-dihydroxy-20-methyl-prosta-5(c),13(t)-dienoate.

PREPARATION 5.

This preparation illustrates methods for preparing methyl *dl*-9α-hydroxy-15-oxo-20-ethyl-prost-13(t)-enoate (VII, 9α—OH, $R_1=C_2H_5$, $Z=CH_2CH_2$). In this preparation, a solution of 2.006 g of methyl *dl*-9α,15α-dihydroxy-20-ethyl-prost-13(t)-enoate, prepared as described in Preparation 4, in 100 ml of benzene is stirred with 3.5 g of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone for 36 hours at room temperature. The reaction mixture is then diluted with 100 ml of benzene, washed with 100 ml of 5% aqueous sodium bisulfite, 200 ml of saturated aqueous sodium bicarbonate and dried over anhydrous sodium sulfate. The benzene solution is concentrated *in vacuo* and the residue chromatographed on 300 g of silica gel. Elution with 20% ethyl acetate/hexane (v/v) yields 1.188 g of methyl *dl*-9α-hydroxy-15-oxo-20-ethyl-prost-13(t)-enoate.

In a similar manner, by substituting methyl *dl*-9α,15β-dihydroxy-20-ethyl-prost-13(t)-enoate for methyl *dl*-9α,15α-dihydroxy-20-ethyl-prost-13(t)-enoate, methyl *dl*-9α-hydroxy-15-oxo-20-ethyl-prost-13(t)-enoate is obtained.

Similarly, by substituting
methyl *dl*-9β,15α- or 9β,15β-dihydroxy-20-ethyl-prost-13(t)-enoate or
methyl *dl*-9α,15α- or 9α,15β-dihydroxy-prost-13(t)-enoate or
methyl *dl*-9β,15α- or 9β,15β-dihydroxy-prost-13(t)-enoate or
methyl *dl*-9α,15α- or 9α,15β-dihydroxy-20-methyl-prost-13(t)-enoate or
methyl *dl*-9β,15α- or 9β,15β-dihydroxy-20-methyl-prost-13(t)-enoate or
methyl *dl*-9α,15α- or 9α,15β-dihydroxy-20-ethyl-prosta-5(c),13(t)-dienoate or
methyl *dl*-9β,15α- or 9β,15β-dihydroxy-20-ethyl-prosta-5(c),13(t)-dienoate or
methyl *dl*-9α,15α- or 9α,15β-dihydroxy-prosta-5(c),13(t)-dienoate or
methyl *dl*-9β,15α- or 9β,15β-dihydroxy-prosta-5(c),13(t)-dienoate or
methyl *dl*-9α,15α- or 9α,15β-dihydroxy-20-methyl-prosta-5(c),13(t)-dienoate or
methyl *dl*-9β,15α- or 9β,15β-dihydroxy-20-methyl-prosta-5(c),13(t)-dienoate
respectively, for methyl *dl*-9α,15α-dihydroxy-20-ethyl-prost-13(t)-enoate in the reaction, the following compounds of formula VII are obtained:

methyl *dl*-9β-hydroxy-15-oxo-20-ethyl-prost-13(t)-enoate;
methyl *dl*-9α-hydroxy-15-oxo-prost-13(t)-enoate;
methyl *dl*-9β-hydroxy-15-oxo-prost-13(t)-enoate;
methyl *dl*-9α-hydroxy-15-oxo-20-methyl-prost-13(t)-enoate;
methyl *dl*-9β-hydroxy-15-oxo-20-methyl-prost-13(t)-enoate;
methyl *dl*-9α-hydroxy-15-oxo-20-ethyl-prosta-5(c),13(t)-dienoate;
methyl *dl*-9β-hydroxy-15-oxo-20-ethyl-prosta-5(c),13(t)-dienoate;
methyl *dl*-9α-hydroxy-15-oxo-prosta-5(c),13(t)-dienoate;
methyl *dl*-9β-hydroxy-15-oxo-prosta-5(c),13(t)-dienoate;
methyl *dl*-9α-hydroxy-15-oxo-20-methyl-prosta-5(c),13(t)-dienoate; and
methyl *dl*-9β-hydroxy-15-oxo-20-methyl-prosta-5(c),13(t)-dienoate;
respectively.

PREPARATION 6.

This preparation illustrates methods for preparing methyl *dl*-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoate (VIII, 9α—OH, 15α—OH, 15β—CH_3, R_1=C_2CH_2), and its isomer methyl *dl*-9α,15β-dihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoate, (VIII, 9α—OH, 15β—OH, 15α—CH_3, R_1=C_2H_5, Z=CH_2CH_2).

In this preparation, a solution of 1.188 g of methyl *dl*-9α-hydroxy-15-oxo-20-ethyl-prost-13(t)-enoate, prepared as described in Preparation 5, in 70 ml of tetrahydrofuran is cooled to −30°C and treated with 6.0 ml of 3 N methylmagnesium bromide in tetrahydrofuran. After stirring for 45 minutes at −30°C, the reaction is quenched by the addition of 3 ml of acetone and then poured into 200 ml of ice water. The aqueous solution is then extracted three times with 65 ml of ethyl acetate and the combined ethyl acetate extracts washed with 300 ml of saturated aqueous sodium chloride. The organic layer is then dried over anhydrous sodium sulfate and concentrated *in vacuo*. The resulting residue is chromatographed on 350 g of silica gel. Elution with 20% ethyl acetate — hexane (v/v) gives 0.511 g of methyl *dl*-9α,15β-dihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoate. Further elution with 25% ethyl acetate — hexane (v/v) yields 0.454 g of methyl *dl*-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoate.

In a similar manner, by substituting
methyl *dl*-9β-hydroxy-15-oxo-20-ethyl-prost-13(t)-enoate;
methyl *dl*-9α-hydroxy-15-oxo-prost-13(t)-enoate;
methyl *dl*-9β-hydroxy-15-oxo-prost-13(t)-enoate;
methyl *dl*-9α-hydroxy-15-oxo-20-methyl-prost-13(t)-enoate;
methyl *dl*-9β-hydroxy-15-oxo-20-methyl-prost-13(t)-enoate;
methyl *dl*-9α-hydroxy-15-oxo-20-ethyl-prosta-5(c),13(t)-dienoate;
methyl *dl*-9β-hydroxy-15-oxo-20-ethyl-prosta-5(c),13(t)-dienoate;
methyl *dl*-9α-hydroxy-15-oxo-prosta-5(c),13(t)-dienoate;
methyl *dl*-9β-hydroxy-15-oxo-prosta-5(c),13(t)-dienoate;
methyl *dl*-9α-hydroxy-15-oxo-20-methyl-prosta-5(c),13(t)-dienoate; and
methyl *dl*-9β-hydroxy-15-oxo-20-methyl-prosta-5(c),13(t)-dienoate for
methyl *dl*-9α-hydroxy-15-oxo-20-ethyl-prost-13(t)-enoate
and following the procedure as described above, the following pair of compounds of formula VIII respectively, are obtained which are separated into their optically pure isomers by thin-layer chromatography;
methyl *dl*-9β,15α-dihydroxy-15β- and 15α-methyl-20-ethyl-prost-13(t)-enoate,
methyl *dl*-9α,15α-dihydroxy-15β- and 15α-methyl-prost-13(t)-enoate,
methyl *dl*-9β,15α-dihydroxy-15β- and 15α-methyl-prost-13(t)-enoate,
methyl *dl*-9α,15α-dihydroxy-15β- and 15α,20-di-methyl-prost-13(t)-enoate,
methyl *dl*-9β,15α-dihydroxy-15β- and 15α,20-di-methyl prost-13(t)-enoate,
methyl *dl* - 9α,15α - dihydroxy-15β- and 15α-methyl-20-ethyl-prosta-5(c),13(t)-dienoate,
methyl *dl*-9β,15α-dihydroxy-15β- and 15α - methyl - 20 - ethyl - prosta - 5(c),13(t)-dienoate,
methyl *dl*-9α,15α-dihydroxy-15β- and 15α-methyl-prosta-5(c),13(t)-dienoate,
methyl *dl*-9β,15α-dihydroxy-15β- and 15α-methyl-prosta-5(c),13(t)-dienoate,
methyl *dl*-9α,15α-dihydroxy-15β- and 15α,20-di-methyl-prosta-5(c),13(t)-dienoate,
methyl *dl*-9β,15α-dihydroxy-15β- and 15α,20-di-methyl-prosta-5(c),13(t)-dienoate.

PREPARATION 7.

This preparation illustrates methods for preparing methyl *dl*-9-oxo-15α-hydroxy - 15β - methyl - 20 - ethyl - prost - 13(t) - enoate (XI, 15α—OH, 15β—CH_3, R_1=C_2H_5, Z=CH_2CH_2).

In this preparation, a suspension of 1.00 g of Celite (diatomaceous earth), 1.60 g of chromium trioxide and 53 ml of anhydrous methylene chloride is stirred under nitrogen while 2.29 g of pyridine are added. The resulting suspension is stirred at room temperature for 30 minutes. A solution of 0.94 g of methyl *dl*-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoate prepared as described in Preparation 6, in 5 ml of methylene chloride is added. After 30 minutes at room temperature, the reaction mixture is filtered through 50 g of alumina. The alumina is washed several times with methylene chloride and the combined filtrates concentrated under reduced pressure to give 0.76 g of methyl *dl*-9-oxo-15α-hydroxy-15β-methyl-20-ethyl-prost-13(t)-enoate.

Similarly, by substituting methyl *dl*-9β,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoate for methyl *dl*-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoate, methyl *dl*-9-oxo-15α-hydroxy-15β-methyl-20-ethyl-prost-13(t)-enoate is obtained.

In a similar manner, by substituting
methyl *dl*-9α,15β- or 9β,15β-dihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoate,
methyl *dl*-9α,15β- or 9β,15β-dihydroxy-15α-methyl-prost-13(t)-enoate,
methyl *dl*-9α,15α- or 9β,15α-dihydroxy-15β-methyl-prost-13(t)-enoate,
methyl *dl*-9α,15β- or 9β,15β-dihydroxy-15α,20-di-methyl-prost-13(t)-enoate,
methyl *dl*-9α,15α- or 9β,15α-dihydroxy-15β,20-di-methyl-prost-13(t)-enoate,
methyl *dl*-9α,15β- or 9β,15β - dihydroxy - 15α - methyl - 20-ethyl-prosta-5(c),13(t)-dienoate,
methyl *dl*-9α,15α- or 9β,15α - dihydroxy - 15β - methyl - 20-ethyl-prosta-5(c),13(t)-dienoate,
methyl *dl*-9α,15β- or 9β,15β - dihydroxy - 15α - methyl - prosta - 5(c),13(t)-dienoate,
methyl *dl*-9α,15α- or 9β,15α-dihydroxy-15β-methyl-prosta-5(c),13(t)-dienoate,
methyl *dl*-9α,15β- or 9β,15β - dihydroxy - 15α,20 - di - methyl - prosta - 5(c),13(t)-dienoate, or
methyl *dl*-9α,15α- or 9β,15α - dihydroxy - 15β,20 - di - methyl - prosta - 5(c),13(t)-dienoate
respectively for
methyl *dl*-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoate
as starting material and following the procedure described above, the compounds of formula IX listed below are obtained:
methyl *dl*-9-oxo-15β-hydroxy-15α-methyl-20-ethyl-prost-13(t)-enoate;
methyl *dl*-9-oxo-15β-hydroxy-15α-methyl-prost-13(t)-enoate;
methyl *dl*-9-oxo-15α-hydroxy-15β-methyl-prost-13(t)-enoate;
methyl *dl*-9-oxo-15β-hydroxy-15α,20-di-methyl-prost-13(t)-enoate;
methyl *dl*-9-oxo-15α-hydroxy-15β,20-di-methyl-prost-13(t)-enoate;
methyl *dl*-9-oxo-15β-hydroxy-15α-methyl-20-ethyl-prosta-5(c),13(t)-dienoate;
methyl *dl*-9-oxo-15α-hydroxy-15β-methyl-20-ethyl-prosta-5(c), 13(t)-dienoate;
methyl *dl*-9-oxo-15β-hydroxy-15α-methyl-prosta-5(c),13(t)-dienoate;
methyl *dl*-9-oxo-15α-hydroxy-15β-methyl-prosta-5(c),13(t)-dienoate;
methyl *dl*-9-oxo-15β-hydroxy-15α,20-di-methyl-prosta-5(c),13(t)-dienoate; and
methyl *dl*-9-oxo-15α-hydroxy-15β,20-di-methyl-prosta-5(c),13(t)-dienoate;
respectively.

PREPARATION 8.

This preparation illustrates methods for preparing *dl*-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid, (VIII, 9α—OH, 15α—OH, 15β—CH_3, free acid, $R_1=C_2H_5$, $Z=CH_2CH_2$).

In this preparation, a solution of 0.454 g of methyl *dl*-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoate, prepared as described in Preparation 6, 0.75 g of potassium hydroxide, 10 ml of methanol and 10 ml of water is stirred at room temperature under nitrogen for 1.75 hours. The reaction mixture is diluted with 50 ml of water and washed with 100 ml of diethyl ether. The aqueous layer is then acidified to pH 4 with 1 N hydrochloric acid, saturated with sodium chloride, and extracted three times with 75 ml of ethyl acetate. The combined ethyl acetate extracts are washed with 300 ml of saturated aqueous sodium chloride and dried over anhydrous sodium sulfate. Concentration of the organic solution gives a residue which is recrystallized from 1 ml of ethyl acetate and 10 ml of hexane. On cooling overnight at −20°C, 0.329 g of *dl*-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid precipitates and is collected by filtration.

Similarly, by substituting the other compounds obtained in Preparation 6 for

methyl dl-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoate and following the procedure as described above, the following free acids, corresponding to compounds of formula VIII are obtained:
dl-9α,15β-dihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid;
dl-9β,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid;
dl-9β,15β-dihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid;
dl-9α,15α-dihydroxy-15β-methyl-prost-13(t)-enoic acid;
dl-9α,15β-dihydroxy-15α-methyl-prost-13(t)-enoic acid;
dl-9β,15α-dihydroxy-15β-methyl-prost-13(t)-enoic acid;
dl-9β,15β-dihydroxy-15α-methyl-prost-13(t)-enoic acid;
dl-9α,15α-dihydroxy-15β,20-di-methyl-prost-13(t)-enoic acid;
dl-9α,15β-dihydroxy-15α,20-di-methyl-prost-13(t)-enoic acid;
dl-9β,15α-dihydroxy-15β,20-di-methyl-prost-13(t)-enoic acid;
dl-9β,15β-dihydroxy-15α,20-di-methyl-prost-13(t)-enoic acid;
dl-9α,15α-dihydroxy-15β-methyl-20-ethyl-prosta-5(c),13(t)-dienoic acid;
dl-9α,15β-dihydroxy-15α-methyl-20-ethyl-prosta-5(c),13(t)-dienoic acid;
dl-9β,15α-dihydroxy-15β-methyl-20-ethyl-prosta-5(c),13(t)-dienoic acid;
dl-9β,15β-dihydroxy-15α-methyl-20-ethyl-prosta-5(c),13(t)-dienoic acid;
dl-9α,15α-dihydroxy-15β-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9α,15β-dihydroxy-15α-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9β,15α-dihydroxy-15β-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9β,15β-dihydroxy-15α-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9α,15α-dihydroxy-15β,20-di-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9α,15β-dihydroxy-15α,20-di-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9β,15α-dihydroxy-15β,20-di-methyl-prosta-5(c),13(t)-dienoic acid; and
dl-9β,15β-dihydroxy-15α,20-di-methyl-prosta-5(c),13(t)-dienoic acid.

In a similar manner, by substituting the compounds prepared in Preparation 7 for methyl dl-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoate and following the procedure as described above, the following free acids, corresponding to compounds of formula IX are obtained:
dl-9-oxo-15α-hydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid;
dl-9-oxo-15β-hydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid;
dl-9-oxo-15α-hydroxy-15β-methyl-prost-13(t)-enoic acid;
dl-9-oxo-15β-hydroxy-15α-methyl-prost-13(t)-enoic acid;
dl-9-oxo-15α-hydroxy-15β,20-di-methyl-prost-13(t)-enoic acid;
dl-9-oxo-15β-hydroxy-15α,20-di-methyl-prost-13(t)-enoic acid;
dl-9-oxo-15α-hydroxy-15β-methyl-20-ethyl-prosta-5(c),13(t)-dienoic acid;
dl-9-oxo-15β-hydroxy-15α-methyl-20-ethyl-prosta-5(c),13(t)-dienoic acid;
dl-9-oxo-15α-hydroxy-15β-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9-oxo-15β-hydroxy-15α-methyl-prosta-5(c),13(t)-dienoic acid;
dl-9-oxo-15α-hydroxy-15β,20-di-methyl-prosta-5(c),13(t)-dienoic acid; and
dl-9-oxo-15β-hydroxy-15α,20-di-methyl-prosta-5(c),13(t)-dienoic acid.

Also, by substituting the compounds prepared in Preparation 4 for methyl dl-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoate and following the procedure as described above, the following free acids, corresponding to compounds of formula VI are obtained:
dl-9α,15α- 9β,15α-, 9α,15β- and 9β,15β-dihydroxy-20-ethyl-prost-13(t)-enoic acid;
dl-9α,15α-, 9β,15α-, 9α,15β- and 9β,15β-dihydroxy-prost-13(t)-enoic acid;
dl-9α,15α-, 9β,15α-, 9α,15β- and 9β,15β-dihydroxy-20-methyl-prost-13(t)-enoic acid;
dl-9α,15α-, 9β,15α-, 9α,15β- and 9β,15β-dihydroxy-20-ethyl-prosta-5(c),13(t)-dienoic acid;
dl-9α,15α-, 9β,15α-, 9α,15β- and 9β,15β-dihydroxy-prosta-5(c),13(t)-dienoic acid and
dl-9α,15α-, 9β,15α-, 9α,15β- and 9β,15β-dihydroxy-20-methyl-prosta-5(c),13(t)-dienoic acid.

Similarly, by substituting the compounds prepared in Preparation 3 for methyl dl-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoate and following the procedure as described above, the following free acids, corresponding to compounds of formula V are obtained:
dl-9-oxo-15α- and 15β-hydroxy-20-ethyl-prost-13(t)-enoic acid;
dl-9-oxo-15α- and 15β-prost-13(t)-enoic acid
dl-9-oxo-15α- and 15β-hydroxy-20-methyl-prost-13(t)-enoic acid;
dl-9-oxo-15α- and 15β-hydroxy-20-ethyl-prosta-5(c),13(t)-dienoic acid;

dl-9-oxo-15α- and 15β-hydroxy-prosta-5(c),13(t)-dienoic acid; and
dl-9-oxo-15α- and 15β-hydroxy-20-methyl-prosta-5(c),13(t)-dienoic acid.

In this Specification use is made of the microbiological classification according to the scheme proposed by Ainsworth (1966): "A general purpose classification of fungi — Bibliography of Systematic Mycology (1966), 1—4 — Commonwealth Mycological Institute — Kew, Surrey", and use is made of Ainsworth and Bisby's Dictionary of the Fungi, 6th edition (1971).

The aforesaid Division of Eumycota embraces 5 Sub-divisions, *viz.* Mastigomycotina, Deuteromycotina, Basidiomycotina, Ascomycotina and Zygomycotina. While numerous species of microorganisms falling within the 5 Sub-divisions of Eumycota can be employed in the process of the invention for the preparation of the 18ξ, 19ξ- and 20ξ-hydroxy-prostaglandin derivatives of general formula I, it is preferred to employ species of microorganisms falling within the Classes and Orders listed herebelow:

MASTIGOMYCOTINA
- *Oomycetes*
  - Saprolegniales
  - Peronosporales

DEUTEROMYCOTINA
- *Coelomycetes*
  - Sphaeropsidales
  - Melanconiales
- *Hyphomycetes*
  - Hyphomycetales
  - Tuberculariales

BASIDIOMYCOTINA
- *Gasteromycetes*
  - Lycoperdales
- *Hymenomycetes*
  - Aphyllophorales
  - Agaricales

ASCOMYCOTINA
- *Plectomycetes*
  - Eurotiales
  - Microascales
- *Pyrenomycetes*
  - Sphaeriales
  - Hypocreales
- *Loculoascomycetes*
  - Pleosporales

ZYGOMYCOTINA
- *Zygomycetes*
  - Mucorales
  - Entomophthorales

While numerous species of microorganisms falling within the Family of *Streptomycetaceae* can be employed in the process of the invention for the preparation of the 18ξ- and 19ξ-hydroxy-prostaglandin derivatives of general formula I, it is preferred to employ species of microorganisms falling within the genus Streptomyces.

Cultures of a large number of species, falling within the group of microorganisms which can be employed in the process of the invention, are available from known sources, such as:

"Centraal Bureau voor Schimmelcultures" (CBS), Baarn, The Netherlands;
"American Type Culture Collection" (ATCC), Rockville, Maryland, U.S.A.;
"Northern Utilization Research and Development Division of U.S. Department of Agriculture" (NRRL), Peoria, Illinois, U.S.A. and
"Commonwealth Mycological Institute" (CMI), Kew, Surrey, England.

The microorganism to be used is grown in the conventional way, preferably in a liquid medium with constant aeration by shaking or by stirring and aerating. Culture media used for the growth of fungal organisms and Streptomyces are well known in the art and principally consist of (1) a source of carbon such as glucose, maltose, sucrose, starch, dextrine and vegetable oils and (2) a source of nitrogen such as ammonia salts, meat and fish flours, corn steep solids and other nutritive substances containing nitrogen, (3) inorganic salts such as sodium, potassium, magnesium, sulphates, phosphates and chlorides, and, optionally, trace elements. The foregoing materials are added in the desired amounts to a quantity of tap water, and the solution is sterilised prior to inoculation with the microorganism culture.

The prostaglandin or prostaglandin derivative of general formula II to be hydroxylated can be added in the form of a fine crystal suspension or dissolved in a solvent such as acetone, ethanol or dimethyl formamide. During the incubation of the starting prostaglandin with the fungus or streptomycete cultures, aeration may be provided by shaking and the temperature is normally kept between 20 and 40°C for 12—48 hours. The hydroxylation can be followed by thin-layer chromatography. The hydroxylated products can be isolated from the fermentation broth by known procedures. At the end of the fermentation, the broth can be filtered, the filtrate acidified to about pH 3 and extracted with a suitable organic solvent. For acidification organic or mineral acids can be used, such as phosphoric acid, sulphuric acid, formic acid, and citric acid. Extraction can be carried out at pH between 1 and 5. However, it is advisable not to work at pH lower than 2, as many prostaglandin derivatives are acid sensitive. Suitable solvents for extraction are ketones, esters and ethers, such as methyl isobutyl ketone, ethyl acetate and diethyl ether. It is also possible to acidify the culture broth and extract directly without filtration.

The crude products may be purified by known procedures such as direct crystallisation or column chromatography. A suitable adsorbent is, for example, silica gel. The silica is normally pre-treated with 20% of water containing 1% of acetic acid and the column eluted with suitable organic solvents or mixtures thereof, such as ethyl acetate — heptane (8:3 v/v) containing 0.1% by vol.) of acetic acid.

The analysis of the resulting products sometimes presents some difficulty. Mass spectrometry of prostaglandins often yields complex spectra, which are difficult to interpret. Sometimes even the molecular peak cannot be determined.

Better results are obtained by protecting reactive groups such as hydroxyl groups, oxo groups and carboxylic groups by the following reactions:

1. esterification of the carboxylic groups with diazomethane;
2. transformation of oxo groups into methoximes; and
3. conversion of hydroxyl groups into trimethylsilyloxy groups, for example with *N,O*-bis(trimethylsilyl)trifluoroacetamide.

Such converted products are hereinafter referred to as "protected products". The crude derivative can then be injected into a GLC-column connected to a double focussing mass spectrometer and the spectrum of the largest GLC-peak is recorded. GLC is used to obtain a separation of main products from byproducts and to record C-values according to the method of S. Bergström et al., J. Biol. Chem. —*238* (1963), 3555.

For the determination of these values mixtures of normal-fatty acids are used as standards. The retention times of the standards are plotted on a logarithmic scale against the number of carbon atoms of the acids on a linear scale. These diagrams are then used to convert observed retention times to C-values.

These C-values are obtained using the following gas chromatographic conditions:

Column: 5 ft, 2.3 mm i.d.
Stationary phase: 3% OV-17 on Gaschrom Q 100—120 mesh
Oven temperature: 235°C
Carrier gas: 38 ml N_2/min.

The 18ξ-hydroxy and 19ξ-hydroxy-prostaglandin derivatives are usually obtained as a mixture; the isomers can be separated from each other and each of the isomers isolated according to the procedures described above. Sometimes 17ξ-hydroxylated products are also obtained as byproducts. These 17ξ-hydroxy-prostaglandin derivatives are also novel compounds. The hydroxylation of PGA_2 is usually preceded by reduction of the 10(11) double bond.

The alkyl esters of the invention can be obtained by treatment of the

compounds of general formula I with an excess of a diazoalkane such as diazomethane, diazoethane or diazopropane for example, in diethyl ether or methylene chloride solution, in a conventional manner.

Alternatively, the mixture of 18ξ- and 19ξ-hydroxylated compounds can be esterified as described immediately above, and the 18ξ-hydroxy and 19ξ-hydroxyalkyl esters recovered, purified and/or separated, according to procedures described above for the compounds of formula I.

The salts of the invention can be prepared by treating the corresponding free acids of formula I with about one molar equivalent of a suitable base, such as sodium hydroxide, potassium hydroxide, ammonium hydroxide, calcium hydroxide, trimethylamine, triethylamine, tripropylamine, β-(dimethylamino) ethanol, β-(diethylamino) ethanol, triethanolamine, arginine, lysine, caffeine, or procaine. The reaction is usually conducted in an aqueous solution, alone or in combination with an inert, water-miscible organic solvent, at a temperature of from 0°C to 30°C, preferably at room temperature. Typical inert, water-miscible organic solvents which can be used include methanol, ethanol, isopropanol, butanol, dioxane or tetrahydrofuran. When divalent metal salts are prepared, such as the calcium salts or magnesium salts, the free acid starting material is treated with at least one half molar equivalent of the base.

The free acids, alkyl esters or salts of the 18ξ-, 19ξ- and 20ξ-hydroxyprostaglandin derivatives of general formula I or of general formula IA when prepared by the process described above can be administered in a wide variety of dosage forms, either alone or in combination with other pharmaceutical compatible medicaments, in the form of pharmaceutical compositions suited for oral or parenteral administration or inhalation. Such compositions in which a formula I compound or a formula IA compound when obtained by the process described above, is formulated with a pharmaceutically acceptable carrier comprise a further aspect of the present invention. The compounds are typically administered as pharmaceutical compositions consisting essentially of the free acids, alkyl esters or salts of the invention, and a pharmaceutical carrier. The pharmaceutical carrier can be either a solid material, liquid or aerosol, in which the free acid, alkyl ester or salt is dissolved, dispersed or suspended, and can optionally contain small amounts of preservatives and/or pH-buffering agents. Suitable preservatives which can be used include, for example, benzyl alcohol. Suitable buffering agents include, for example, sodium acetate and pharmaceutically acceptable phosphate salts.

The liquid compositions can, for example, be in the form of solutions, emulsions, suspensions, syrups, or elixirs. The solid compositions can take the form of tablets, powders, capsules, or pills, preferably in unit dosage forms for simple administration or precise dosages. Suitable solid carriers include, for example, pharmaceutical grades of starch, lactose, sodium saccharin, talcum, or sodium bisulfite.

For inhalation administration, the free acids, alkyl esters or salts can, for example, be administered as an aerosol in an inert propellant together with a cosolvent, e.g. ethanol, together with optional preservatives, surfactants, stabilisers, isotonic and buffering agents. Additional general information concerning the inhalation administration of aerosols can be had by reference to U.S. Patent Specification Nos. 2,868,691 and 3,095,355.

For the preparation of an aerosol the active compound is first micronised; preferred particle size is from 0.5 to 10 μ. The solutions or suspensions to be used contain from 0.02 to 0.5 mg of active compound per ml of pharmaceutically acceptable solvent medium. Preferably, the pH of the solution or suspension is between 4 and 7.

The solutions or suspensions are used in an aerosol container provided with a metered valve which releases preferably from 50 to 60 μl per puff. Propellants conventional in pharmaceutical aerosols, such as various chloro-fluoro-alkanes, may be used.

\ suitable aerosol can be prepared, for example, using solutions or suspensions and propellants consisting of:

9-oxo-11α,15α,19ξ-trihydroxy-prost-13(t)-enoic acid triethanolamine salt	0.25%
ethanol absolute	36.75%
dichlorodifluoromethane/1,2-dichloro-1,1,2,2-tetrafluoroethane (40/60 v/v) ad	100%
or	
9-oxo-11α,15α,18ξ-trihydroxy-prost-13(t)-enoic acid	0.5 g
propylene glycol	1 g
ethanol absolute	19.5 g
dichlorodifluoromethane/1,2-dichloro-1,1,2,2-tetrafluoroethane (40/60 v/v) ad	100 g
optionally together with preservatives, surfactants, stabilisers, isotonic and buffering agents.	

The free acids, alkyl esters or salts of the invention are typically administered i.v. in dosages of 0.1 to 10 mg and *p.o.* in dosages of 1 to 100 mg. The daily doses are *i.v.* 0.4 to 40 mg and *p.o.* 6 to 600 mg.

The following Examples illustrate the invention.

EXAMPLE 1.

a. An agar slant of *Thozetellopsis tocklaiensis* (CBS 378.58) was used to inoculate 100 ml of sterile 20—20 medium in a 500 ml conical flask. This medium was prepared by solving 20 g of glucose in 500 ml of tap water, adding 20 g of corn steep solids and making up to 1 litre with tap water; pH was adjusted to 6.5 with the aid of a 30% (w/v) solution of sodium hydroxide. Sterilization was carried out for 20 minutes at 120°C.

The flask was incubated for 72 hours at 26°C on a rotary shaker (280 r.p.m., 2.5 cm stroke). From the resulting culture, 5 ml were used to inoculate 100 ml of sterile 10—10 medium in a 500 ml conical flask. The medium was prepared as the 20—20 medium described above using 10 g of glucose and corn steep solids each per litre. The flask was incubated at 26°C on the rotary shaker.

18 Hours after inoculation, 20 mg of *dl*-9-oxo-15α-hydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 3 and 8, dissolved in 2.5 ml of 50% (v/v) aqueous ethanol, were added and the incubation was continued for another 24 hours at 26°C. The culture broth was then filtered, the filtrate acidified to pH 3 with a 10% (w/v) aqueous citric acid solution, and extracted three times with 20 ml of ethyl acetate. The extract was evaporated *in vacuo* and the residue purified by column chromatography (SiO_2 pretreated with 1% (v/v) acetic acid; eluted with ethyl acetate — heptane (8:3 v/v) containing 0.1% by vol. acetic acid). The matching fractions were combined and evaporated *in vacuo* to give 2.5 mg of 9-oxo-15α,18ξ-dihydroxy-prost-13(t)-enoic acid and 3.5 mg of 9-oxo-15α,19ξ-dihydroxy-prost-13(t)-enoic acid.

The protected 18-hydroxy product (silyl ether, methoxime, methyl ester) has:
C-value: 25.9
Molecular peak in mass spectrum: m/e=541
Intense peaks: 510, 420, 382, 309, 197, 131, 129.
Minor characteristic fragments: 422, 390, 364, 222, 144.

The protected 19-hydroxy product has:
C-value: 26.2
Molecular peak in mass spectrum: m/e=541
Intense peaks: 510, 420, 382, 129, 117.
Minor characteristic fragments: 466, 368, 330, 309, 222, 143.

b. In a similar way, *dl*-9-oxo-15β-hydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 3 and 8, was converted into 9-oxo-15β,18ξ-dihydroxy-

prost-13(t)-enoic acid and 9-oxo-15β,19ξ-dihydroxy-prost-13(t)-enoic acid.

The protected 18-hydroxy product has:
C-value: 26.0
Molecular peak in mass spectrum: m/e=541
Intense peaks: 510, 420, 382, 309, 197, 131, 129.
Minor characteristic fragments: 422, 390, 364, 222, 144.

The protected 19-hydroxy product has:
C-value: 26.3
Molecular peak in mass spectrum: m/e=541
Intense peaks: 510, 420, 382, 129, 117.
Minor characteristic fragments: 466, 368, 330, 309, 222, 143.

c. In a similar way, *dl*-9α,15β-dihydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 4 and 8, was converted into 9α,15β,18ξ-trihydroxy-prost-13(t)-enoic acid and 9α,15β,19ξ-trihydroxy-prost-13(t)-enoic acid. The silylated methyl ester of the 18-hydroxy compound has:
C-value: 24.3
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 297, 197, 131, 129.
Minor characteristic fragments: 557, 496, 467, 377, 350, 310, 247, 144.

The silylated methyl ester of the 19-hydroxy compound has:
C-value: 24.6
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 297, 197, 143, 129, 117.
Minor characteristic fragments: 496, 452, 310, 247, 143.

d. In a similar way, *dl*-9β,15β-dihydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 4 and 8, was converted into 9β,15β,18ξ-trihydroxy-prost-13(t)-enoic acid and 9β,15β,19ξ-trihydroxy-prost-13(t)-enoic acid. The silylated methyl ester of the 18-hydroxy compound has:
C-value: 24.3
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 247, 197, 131, 129.
Minor characteristic fragments: 557, 467, 377, 350, 297, 223.

The silylated methyl ester of the 19-hydroxy compound has:
C-value: 24.6
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 247, 197, 129, 117.
Minor characteristic fragments: 452, 297, 223.

e. In a similar way, 9-oxo-15α-hydroxy-prosta-5(c),8(12),13(t)-trienoic acid (PGB_2) was converted into 9-oxo-15α,18ξ-dihydroxy-prost-5(c),8(12),13(t)-trienoic acid and 9-oxo-15α,19ξ-dihydroxy-prosta-5(c),8(12),13(t)-trienoic acid. The protected 18-hydroxy compound (silyl ether, methyl ester, methoxime) has:
C-value: 27.2.
Molecular peak in mass spectrum: m/e=537
Intense peaks: 506, 416, 360, 131.
Minor characteristic fragments: 418, 378, 326, 162.

The protected 19-hydroxy derivative has:
C-value: 27.7
Molecular peak in mass spectrum: m/e=537
Intense peaks: 506, 416, 129, 117.
Minor characteristic fragments: 378, 346, 326, 162.

f. In a similar way, 9-oxo-15α-hydroxy-prosta-5(c),10,13(t)-trienoic acid (PGA_2) was converted into 9-oxo-15α,18ξ-dihydroxy-prosta-5(c),13(t)-dienoic acid and 9-oxo-15α,19ξ-dihydroxy-prosta-5(c),13(t)-dienoic acid.

The protected 18-hydroxy compound has:
C-value: 25.9
Molecular peak in the mass spectrum: m/e=539
Intense peaks: 508, 418, 131, 129.
Minor characteristic fragments: 438, 380, 226, 220, 197.

The protected 19-hydroxy derivative has:
C-value: 26.2
Molecular peak in mass spectrum: m/e=539.
Intense peaks: 508, 418, 380, 348, 143, 129, 117.
Minor characteristic fragments: 438, 226, 220.

g. In a similar way, *dl*-9β,15α-dihydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 4 and 8, was converted into 9β,15α,18ξ-trihydroxy-prost-13(t)-enoic acid and 9β,15α,19ξ-trihydroxy-prost-13(t)-enoic acid. The silylated methyl ester of the 18-hydroxy compound has:
C-value: 24.3
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 247, 197, 131, 129.
Minor characteristic fragments: 557, 467, 377, 350, 297, 223.

The silylated methyl ester of the 19-hydroxy compound has:
C-value: 24.6
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 247, 197, 129, 117.
Minor characteristic fragments: 452, 297, 223.

h. In a similar way, *dl*-9α,15α-dihydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 4 and 8, was converted into 9α,15α,18ξ-trihydroxy-prost-13(t)-enoic acid, and 9α,15α,19ξ-trihydroxy-prost-13(t)-enoic acid.

The silylated methyl ester of the 18-hydroxy product has:
C-value: 24.2
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 297, 197, 131, 129.
Minor characteristic fragments: 557, 496, 467, 377, 350, 310, 247, 144.

The silylated methyl ester of the 19-hydroxy compound has:
C-value: 24.5
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 297, 197, 143, 129, 117.
Minor characteristic fragments: 496, 452, 310, 247, 143.

EXAMPLE II.

a. An agar slant of *Delacroixia coronata* (CBS 647.68) was used to inoculate 100 ml of sterile 20—20 medium in a 500 ml conical flask. This medium was prepared as described in Example I a.

The flask was incubated for 72 hours at 26°C on a rotary shaker (280 r.p.m., 2.5 cm stroke). From the resulting culture, 5 ml were used to inoculate 100 ml of sterile 10—10 medium in a 500 ml conical flask. The medium was prepared as described in Example I a. The flask was incubated at 26°C on the rotary shaker.

18 Hours after inoculation 20 mg of *dl*-9α,15α-dihydroxy-15β-methyl-prost-13(t)-enoic acid, prepared as described in Preparations 6 and 8, dissolved in 2.5 ml of 50% aqueous ethanol, were added and the incubation was continued for another 24 hours at 26°C. The culture broth was then filtered, the filtrate acidified to pH 3 with a 10% w/v aqueous citric acid solution and extracted three times with 20 ml of ethyl acetate. The extract was evaporated *in vacuo* and the residue purified by column chromatography (SiO_2 pretreated with 1% v/v acetic acid; eluted with ethyl acetate — heptanol (8:3 v/v) containing 0.1% by vol acetic acid). The matching fractions were combined and evaporated *in vacuo* to give 2.0 mg of 9α,15α,18ξ-trihydroxy-15β-methyl-prost-13(t)-enoic acid and 3.8 mg of 9α,15α,19ξ-trihydroxy-15β-methyl-prost-13(t)-enoic acid.

The silylated methyl ester of the 18-hydroxy product has:
C-value: 24.2
Molecular peak in mass spectrum: m/e=600
Intense peaks: 441, 351, 297, 211, 143, 131.
Minor characteristic fragments: 585, 571, 481, 323, 301, 257, 144.

The protected 19-hydroxy product has:
C-value: 24.6
Molecular peak in mass spectrum: m/e=600
Intense peaks: 441, 351, 297, 143, 117.
Minor characteristic fragments: 585, 323, 301, 211.

b. In a similar way, *dl*-9-oxo-15β-hydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid, prepared as described in Preparations 7 and 8, was converted into 9-oxo-15β,18ξ-dihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid and 9-oxo-15β,19ξ-dihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid.

The protected 18-hydroxy product (silyl ether, methoxime, methyl ester) has:
C-value: 27.0
Molecular peak in mass spectrum: m/e=583

Intense peaks: 396, 143.
Minor characteristic fragments: 526, 462, 436, 366, 364, 171, 159.

The protected 19-hydroxy product has:
C-value: 27.4
Molecular peak in mass spectrum: m/e=583
Intense peaks: 396, 171, 145, 143.
Minor characteristic fragments: 462, 450, 366, 364, 239.

EXAMPLE III.

a. An agar slant of *Streptomyces* sp. (CBS 188.74) was used to inoculate 100 ml of the following medium in a 500 ml conical flask: peptone 10 g/l, malt paste 15 g/l, NaCl 5 g/l, distilled water; the pH was adjusted to 7.2 with the aid of 30% w/v aqueous potassium hydroxide solution. Sterilization was carried out for 20 minutes at 120°C.

The flask was incubated for 72 hours at 26°C on a rotary shaker (280 r.p.m., 2.5 cm stroke). From the resulting culture, 5 ml were used to inoculate 100 ml of the following medium in a 500 ml conical flask: glucose 10 g/l, corn steep solids 3 g/l, peptone 5 g/l, NaCl 5 g/l, tap water; the pH was adjusted to 7.2 by adding a 30% w/v aqueous potassium hydroxide solution. Sterilization was carried out for 20 minutes at 120°C.

The flask was incubated for 72 hours at 26°C on the rotary shaker. 20 mg of 9-oxo-11α,15α-dihydroxy-prost-13(t)-enoic acid (PGE_1), dissolved in 2.5 ml of 50% v/v aqueous ethanol, were then added and the incubation was continued for another 24 hours. Thin layer chromatography indicated that two compounds were formed which were more polar than the starting material. The fermentation broth was filtered, the filtrate acidified to pH 3 with a 10% w/v aqueous citric acid solution, and extracted three times with 30 ml of ethyl acetate. The extract was evaporated under reduced pressure and the residue purified by column chromatography (SiO_2 pretreated with 1% by vol. acetic acid and 19% by vol. water; eluted with ethyl acetate containing 0.1% by vol. acetic acid). The matching fractions were combined and evaporated under reduced pressure. The less polar of the two transformation products was obtained in 5.0 mg yield as an oil and proved to be 9-oxo-11α,15α,18ξ-trihydroxy-prost-13(t)-enoic acid, according to combined GLC-mass spectrometry. The protected product has:
C-value: 26.6
Molecular peak in mass spectrum: m/e=629
Intense peaks: 297, 133, 131, 129.
Minor characteristic fragments: 598, 510, 470, 420, 380, 366, 310, 223, 197, 144.

The more polar of the transformation products was also obtained as an oil (yield 4 mg). This compound proved to be 9-oxo-11α,15α,19ξ-trihydroxy-prost-13(t)-enoic acid, according to combined GLC-mass spectrometry.

The protected compound has:
C-value: 27.0
Molecular peak in mass spectrum: m/e=629
Intense peaks: 366, 297, 223, 183, 143, 133, 129, 117.
Minor characteristic fragments: 598, 470, 380, 197.

b. In a similar way, 9-oxo-11α,15α-dihydroxy-prosta-5(c),13(t)-dienoic acid (PGE_2) was converted into:
9-oxo-11α,15α,18ξ-trihydroxy-prosta-5(c),13(t)-dienoic acid and
9-keto-11α,15α,19ξ-trihydroxy-prosta-5(c),13(t)-dienoic acid.

The protected 18-hydroxy compound has:
C-value: 26.6
Molecular peak in mass spectrum: m/e=627
Intense peaks: 596, 506, 366, 295, 223, 133, 131, 129.
Minor characteristic fragments: 508, 468, 418, 378, 364, 197, 144.

The protected 19-hydroxy compound has:
C-value: 26.9
Molecular peak in mass spectrum: m/e=627
Intense peaks: 596, 506, 366, 295, 223, 143, 133, 129, 117.
Minor characteristic fragments: 468, 378, 364, 197.

c. In a similar way, 9α,11α,15α-trihydroxy-prosta-5(c),13(t)-dienoic acid ($PGF_{2\alpha}$) was converted into 9α,11α,15α,18ξ-tetrahydroxy-prosta-5(c),13(t)-dienoic acid and 9α,11α,15α,19ξ-tetrahydroxy-prosta-5(c),13(t)-dienoic acid.

The protected 18-hydroxy product has:
C-value: 25.0
Molecular peak in mass spectrum: m/e=672
Intense peaks: 423, 333, 307, 217, 197, 191, 171, 131, 129.
Minor characteristic fragments: 643, 582, 553, 513, 481, 397.
The protected 19-hydroxy product has:
C-value: 25.3
Molecular peak in mass spectrum: m/e=672
Intense peaks: 423, 333, 307, 217, 197, 191, 143, 129, 117.
Minor characteristic fragments: 657, 582, 567, 531, 513, 481, 397.

d. In a similar way, *dl*-9α,15α-dihydroxy-20-ethyl-prost-13(t)-enoic acid, prepared as described in Preparations 4 and 8, was converted into 9α,15α,18ξ-trihydroxy-20-ethyl-prost-13(t)-enoic acid and 9α,15α,19ξ-trihydroxy-20-ethyl-prost-13(t)-enoic acid.
The protected 18-hydroxy product has:
C-value: 25.6
Molecular peak in mass spectrum: m/e=614
Intense peaks: 427, 337, 297, 129.
Minor characteristic fragments: 557, 467, 377, 225, 159.
The protected 19-hydroxy compound has:
C-value: 25.9
Molecular peak in mass spectrum: m/e=614
Intense peaks: 427, 337, 297, 129.
Minor characteristic fragments: 571, 481, 391, 225, 145.

e. In a similar way, *dl*-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid, prepared as described in Preparations 6 and 8, was converted into 9α,15α,18ξ-trihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid and 9α,15α,19ξ-trihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid.
The protected product has:
C-value: 25.3
Molecular peak in mass spectrum: m/e=628
Intense peaks: 441, 351, 297, 159, 143.
Minor characteristic fragments: 571, 481, 323, 239.
The protected 19-hydroxy derivative has:
C-value: 25.9
Molecular peak in mass spectrum: m/e=628
Intense peaks: 441, 351, 297, 145, 143.
Minor characteristic fragments: 585, 495, 323, 239.

f. In a similar way, *dl*-9α,15β-dihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid, prepared as described in Preparations 6 and 8, was converted into 9α,15β,18ξ-trihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid and 9α,15β,19ξ-trihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid.
The protected 18-hydroxy compound has:
C-value: 25.3
Molecular peak in mass spectrum: m/e=628
Intense peaks: 441, 351, 297, 159, 143.
Minor characteristic fragments: 571, 481, 323, 239.
The protected 19-hydroxy product has:
C-value: 25.9
Molecular peak in mass spectrum: m/e=628
Intense peaks: 441, 351, 297, 145, 143.
Minor characteristic fragments: 585, 495, 323, 239.

The fermentation described below with other *Streptomyces* species were all carried out according to the procedure described in Example III; the fermentation with the other microorganisms were carried out according to the procedure described in Example I.

EXAMPLE IV.

a. Fermentation of *dl*-9β,15α-dihydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 4 and 8, with *Ophiobolus graminis* (ATCC 12761) produced a small amount of 9β,15α,17ξ-trihydroxy-prost-13(t)-enoic acid. The main products of these fermentations were the corresponding 18- and 19-hydroxy isomers, which were identical to the products of Example I g.

The silylated methyl ester of the 17-hydroxy compound has:
C-value: 23.7
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 223, 197, 145, 129.
Minor characteristic fragments: 543, 483, 453, 297, 259, 103.

b. In a similar way, *dl*-9-oxo-15β-hydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 3 and 8, when fermented with *Streptomyces* sp. (CBS 190.74) produced a small amount of 9-oxo-15β,17ξ-dihydroxy-prost-13(t)-enoic acid, in addition to the 18- and 19-hydroxy isomers, which were identical to the products of Example I b.

The protected 17-hydroxy product (silyl ether, methyl ester, methoxime) has:
C-Value: 25.3
Molecular peak in mass spectrum: m/e=541
Intense peaks: 420, 382, 366, 250, 197, 145.
Minor characteristic fragments: 498, 438, 408, 259, 103.

c. In a similar way, *dl*-9α,15α-dihydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 4 and 8, when fermented with *Streptomyces aureofaciens* (ATCC 10762) produced the 19-hydroxy derivative as the main product and small amounts of the 18-hydroxy derivative and 9α,15α,17ξ-trihydroxy-prost-13(t)-enoic acid as by-products; the 18-hydroxy and 19-hydroxy derivatives were identical to the products of Example I c. The silylated methyl ester of the 17-hydroxy compound has:
C-value: 23.7
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 297, 197, 145.
Minor characteristic fragments: 543, 483, 453, 247, 103.

d. In a similar way, *dl*-9β,15β-dihydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 4 and 8, when fermented with *Metarrhizium brunneum* (CBS 316.51) produced the 18- and 19-hydroxy derivatives as main products (which were identical to the products of Example I d) and 9β,15β,17ξ-trihydroxy-prost-13(t)-enoic acid as by-product.

The silylated methyl ester of the 17-hydroxy compound has:
C-value: 23.7
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 223, 197, 145, 129.
Minor characteristic fragments: 543, 483, 453, 297, 259, 103.

e. In a similar way, *dl*-9α,15β-dihydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 4 and 8, when fermented with *Streptomyces griseus* (CBS 479.48) produced a small amount of 9α,15β,17ξ-trihydroxy-prost-13(t)-enoic acid, in addition to the 18- and 19-hydroxy isomers, which were identical to the products of Example I h.

The silylated methyl ester of the 17-hydroxy compound has:
C-value: 23.7
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 297, 197, 145.
Minor characteristic fragments: 543, 483, 453, 247, 103.

EXAMPLE V.

a. A culture of *Stemphylium solani* (NRRL 1805) was grown in a 10—10 medium according to the procedure described in Example I a.

18 Hours after inoculation, 20 mg of *dl*-9α,15β-dihydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 4 and 8, dissolved in 2.5 ml of 50% v/v aqueous ethanol were added and the incubation was continued for another 24 hours at 26°C.

According to TLC, a new compound was formed which was more polar than the starting material. The fermentation broth was filtered, the filtrate acidified to pH 3 with a 10% w/v aqueous citric acid solution, and extracted three times with 20 ml of ethyl acetate. The extract was evaporated under reduced pressure and the residue purified by column chromatography (SiO_2 pretreated with 1% by vol. acetic acid; eluted with ethyl acetate — heptane (8:3 v/v)

containing 0.1% by vol. acetic acid). The matching fractions were combined and evaporated under reduced pressure.

There was obtained 7 mg of 9α,15β,20-trihydroxy-prost-13(t)-enoic acid. The silylated methyl ester of this product has:
C-value: 25.5
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 297, 129.
Minor characteristic fragments: 367, 197, 170, 142, 103.

b. In a similar way, *dl*-9β,15β-dihydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 4 and 8, was converted into 9β,15β,20-trihydroxy-prost-13(t)-enoic acid.

The silylated methyl ester of this product has:
C-value: 25.5
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 129.
Minor characteristics fragments: 367, 297, 197, 170, 142, 103.

c. In a similar way, 9-oxo-15α-hydroxy-prosta-5(c),8(12),13(t)-trienoic acid (PGB_2) was converted by *Aspergillus niger* (ATCC 9142) into 9-oxo-15α,20-dihydroxy-prosta-5(c),8(12),13(t)-trienoic acid.

The protected product has:
C-value: 28.5
Molecular peak in mass spectrum: m/e=537
Intense peaks: 506, 416, 378, 162.
Minor characteristic fragments: 436, 246, 232, 184, 103.

d. In a similar way, 9-oxo-15α-hydroxy-prosta-5(c),10,13(t)-trienoic acid (PGA_2) was transformed by *Preussia fleischhakii* (CBS 167.40) into 9-oxo-15α,20-dihydroxy-prosta-5(c),13(t)-dienoic acid.

The protected product has:
C-value: 27.1
Molecular peak in mass spectrum: m/e=539
Intense peaks: 508, 129.
Minor characteristic fragments: 438, 380, 348, 226, 220, 198, 184, 142, 103.

e. In a similar way, *dl*-9-oxo-15α-hydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid, prepared as described in Preparations 7 and 8, was converted by *Preussia fleischhakii* (CBS 167.40) into 9-oxo-15α,20ξ-dihydroxy-15β-methyl-20ξ-ethyl-prost-13(t)-enoic acid.

The protected product has:
C-value: 27.8
Molecular peak in mass spectrum: m/e=583
Intense peaks: 396, 143, 131.
Minor characteristic fragments: 464, 462, 366, 364, 239, 144.

f. In a similar way *dl*-9-oxo-15β-hydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid, prepared as described in Preparations 7 and 8, was transformed by *Preussia fleischhakii* (CBS 167.40) into 9-oxo-15β,20ξ-dihydroxy-15α-methyl-20ξ-ethylprost-13(t)-enoic acid.

The protected product has:
C-value: 27.8
Molecular peak in mass spectrum: m/e=583
Intense peaks: 396, 143, 131.
Minor characteristic fragments: 464, 462, 366, 239.

g. In a similar way, *dl*-9α,15α-dihydroxy-20-ethyl-prost-13(t)-enoic acid, prepared as described in Preparations 4 and 8, was converted into 9α,15α,20ξ-trihydroxy-20ξ-ethyl-prost-13(t)-enoic acid.

The protected product has:
C-value: 26.3
Molecular peak in mass spectrum: m/e=614
Intense peaks: 427, 337, 297, 246, 131, 129.
Minor characteristic fragments: 585, 495, 323, 310, 211.

h. In a similar way, *dl*-9α,15α-dihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic

The protected product has:
C-value: 26.2
Molecular peak in mass spectrum: m/e=628
Intense peaks: 441, 351, 297, 143, 131.
Minor characteristic fragments: 509, 419, 323, 239.

i. In a similar way, *dl*-9α,15β-dihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid, prepared as described in Preparations 6 and 8, was converted into 9α,15β,20ξ-trihydroxy-15α-methyl-20ξ-ethyl-prost-13(t)-enoic acid.

The protected product has:
C-value: 26.3
Molecular peak in mass spectrum: m/e =628
Intense peaks: 441, 351, 297, 143, 131.
Minor characteristic fragments: 509, 419, 323, 239.

j. In a similar way, *dl*-9-oxo-15α-hydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 3 and 8, was converted by *Pythium ultimum* (CBS 296.37) into 9-oxo-15α,20-dihydroxyprost-13(t)-enoic acid.

The protected product has:
C-value: 27.2
Molecular peak in mass spectrum: m/e = 541
Intense peaks: 510, 382, 222, 129.
Minor characteristic fragments: 420, 368, 309, 197, 103.

k. In a similar way, *dl*-9-oxo-15β-hydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 3 and 8, was converted by *Curvularia trifolii* (CBS 210.59) into 9-oxo-15β-dihydroxy-prost-13(t)-enoic acid.

The protected product has:
C-value: 27.4
Molecular peak in mass spectrum: m/e = 541
Intense peaks: 510, 382, 222, 129.
Minor characteristic fragments: 420, 368, 309, 197, 103.

l. In a similar way, *dl*-9β,15α-dihydroxy-prost-13(t)-enoic acid, prepared as described in Preparations 4 and 8, was converted by *Alternaria radicina* (CBS 245.67) into 9β,15α,20-trihydroxy-prost-13(t)-enoic acid.

The protected product has:
C-value: 25.5
Molecular peak in mass spectrum: m/e=586
Intense peaks: 427, 337, 129.
Minor characteristic fragments: 313, 297, 197, 142, 103.

When the mold *Delacroixia coronata* (CBS 647.68) was fermented with the substrates mentioned in Examples V h and V i, the same 20-hydroxy derivatives were obtained, but as by-product only. Main products with this microorganism were then the 19-hydroxy derivatives of these substrates.

EXAMPLE VI.

a. 10 mg of 9-oxo-11α,15α,18ξ-trihydroxy-prost-13(t)-enoic acid, prepared as described in Example III a, were dissolved in 1 ml of methanol. To this solution 4 ml of an ethereal solution of diazomethane (containing 12 g of diazomethane per liter) were added. The reaction was followed by thin layer chromatography (SiO_2, F_{254} Merck; ethyl acetate/heptane/acetic acid/methanol/water= 40/20/4/6/3 v/v/v/v/v). After 30 minutes the reaction was completed. The solvent was evaporated in a stream of nitrogen and methyl 9-oxo-11α,15α,18ξ-trihydroxy-prost-13(t)-enoate was obtained as an oil.

b. 3.7 mg of 9-oxo-11α,15α,19ξ-trihydroxy-prost-13(t)-enoic acid, prepared as described in Example III a, were dissolved in 0.5 ml of ethyl acetate. To the solution was added a solution of 1.5 mg of triethanolamine in 0.5 ml of ethyl acetate. The resulting solution was evaporated to dryness in a stream of nitrogen and then dried in vacuum to constant weight; 9-oxo-11α,15α,19ξ-trihydroxy-prost-13(t)-enoic acid triethanolamine salt was obtained as an oil.

Other microorganisms capable of introducing an 18-, 19- or 20-hydroxy group in the prostaglandin compounds of formula II are, for example:

Aspergillus amstelodami (CBS 521.65)
Aspergillus chevalieri (CBS 414.67)

Aspergillus flavus (CBS 178.74)
Beauveria alba (CBS 348.55)
Botryosphaeria rhodina (CBS 175.26)
Botrytis cinerea (ATCC 12481)
Coprinus bisporus (CBS 184.52)
Coprinus congregatus (CBS 180.51)
Cunninghamella blakesleeana (NRRL 1373)
Cunninghamella echinulata (CBS 229.51)
Curvularia ellisii (CBS 193.62)
Diplodia alni (CBS 200.49)
Drechslera buchloes (CBS 246.49)
Endothiella gyrosa Sacc. (CBS 253.54)
Entomophtora virulenta (CBS 217.66)
Fusarium senitectum (CBS 181.74)
Fusarium ventricosum (CBS 205.31)
Gliocladium viride Matr. (CBS 191.32)
Gongronella butleri (CBS 259.52)
Hormodendrum chaquense (CBS 231.36)
Hypomyces aurantius (CBS 207.29)
Hypoxylon haematostroma (CBS 255.63)
Hypoxylon jecorinum (CBS 258.63)
Isoachlya turoloides (CBS 598.67)
Lycoperdon gemmatum (CBS 182.74)
Microascus cinereus (CBS 300.61)
Microascus cirrosus (CBS 277.34)
Microascus desmosporus (CBS 424.62)
Mycoacia stenodon (CBS 318.54)
Nigrospora sacchari (CBS 290.62)
Nodulisporium verrucosum (CBS 245.29)
Paecilomyces cremeo-roseus (CBS 250.55)
Paecilomyces farinosus (CBS 183.74)
Pellicularia filamentosa (CBS 184.74)
Pestalotia populi-nigrae (CBS 353.51)
Petriella asymmetrica (CBS 297.58)
Petriellidium boydii (CBS 593.73)
Petriellidium ellipsoideum (CBS 418.73)
Physalospora mutila (CBS 302.36)
Physalospora rhodina (CBS 185.74)
Pseudonectria pachysandricola (CBS 501.63)
Rhizopus nigricans (ATCC 6227b)
Sepedonium chrysospermum (CBS 140.23)
Septoria linicola (CBS 502.50)
Sphaeropsis conspersa (CBS 209.25)
Stemphylium consortiale (NRRL 2187)
Thielavia basicola (CBS 540.50)
Thielavia terricola (CBS 165.73)
Verticillium lecanii (CBS 123.42)

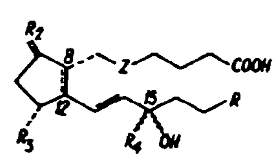
Moreover, an 18- or 19-hydroxy group can also be introduced in the prostaglandin compounds of formula II by various species of the genus *Streptomyces*, for example the species:

Streptomyces chattanoogensis (ATCC 19673)
Streptomyces chattanoogensis (ATCC 13358)
Streptomyces natalensis (CBS 700.57)

and the species with the following CBS deposit numbers: 186.74, 187.74, 189.74, 190.74, 191.74, 192.74, 193.74 and 194.74.

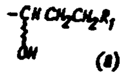
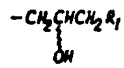
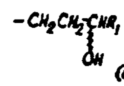
WHAT WE CLAIM IS:—

1. 18ξ-, 19ξ- and 20ξ-Hydroxy-prostaglandin derivatives of the general formula I,



I

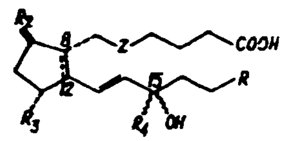
wherein the dotted line in the position 8—12 indicates the optional presence of a double bond, the wavy lines in position 15 indicate that the hydroxyl group and the group R_4 are in either the α- or β-configuration and Z represents a $—CH_2CH_2—$ or a cis —CH=CH— group, and wherein R represents one of the groups:

 (a)  (b) or  (c)

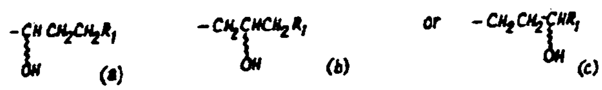
(wherein the wavy lines indicate that the hydroxyl groups are in either the α- or β-configuration and R_1 represents a hydrogen atom, a methyl or ethyl group), R_2 represents either an oxygen atom or a β- or α-hydrogen atom and an α- or β-hydroxyl group, R_3 represents a hydrogen atom or a hydroxyl group and R_4 represents a hydrogen atom or a methyl group, with the proviso that (i) when R_1, R_3 and R_4 each represents a hydrogen atom, R_2 represents an oxygen atom, a double bond is in the 8—12 position and the 15-hydroxyl group is in the α- or β-configuration, R does not represent the group (b), and (ii) when R_1, R_3 and R_4 each represent a hydrogen atom, R_2 represents an oxygen atom, the 15-hydroxyl group is in the α-configuration, Z represents a *cis* — CH=CH — group and the 8—12 position is saturated, R does not represent the group (a), and (iii) when there is a double bond in the 8—12 position, R_3 does not represent a hydroxyl group and (iv) when there is a double bond in the 8—12 position, R_2 does not represent a β- or α-hydrogen and an α- or β-hydroxyl group; and the pharmaceutically acceptable salts and alkyl esters thereof.

2. A compound according to Claim 1, wherein R represents the group (a) or (b).
3. A compound according to Claim 1, wherein R represents the group (c).
4. 9-oxo-15α,18ξ-dihydroxy-prost-13(t)-enoic acid.
5. 9-oxo-15α,19ξ-dihydroxy-prost-13(t)-enoic acid.
6. 9-oxo-15β,18ξ-dihydroxy-prost-13(t)-enoic acid.
7. 9-oxo-15β,19ξ-dihydroxy-prost-13(t)-enoic acid.
8. 9α,15β,18ξ-trihydroxy-prost-13(t)-enoic acid.
9. 9α,15β,19ξ-trihydroxy-prost-13(t)-enoic acid.
10. 9β,15β,18ξ-trihydroxy-prost-13(t)-enoic acid.
11. 9β,15β,19ξ-trihydroxy-prost-13(t)-enoic acid.
12. 9-oxo-15α,18ξ-dihydroxy-prost-5(c),8(12),13(t)-trienoic acid.
13. 9-oxo-15α,19ξ-dihydroxy-prosta-5(c),13(t)-dienoic acid.
14. 9β,15α,18ξ-trihydroxy-prost-13(t)-enoic acid.
15. 9β,15α,19ξ-trihydroxy-prost-13(t)-enoic acid.
16. 9α,15α,18ξ-trihydroxy-prost-13(t)-enoic acid.
17. 9α,15α,19ξ-trihydroxy-prost-13(t)-enoic acid.
18. 9α,15α,18ξ-trihydroxy-15β-methyl-prost-13(t)-enoic acid.
19. 9α,15α,19ξ-trihydroxy-15β-methyl-prost-13(t)-enoic acid.
20. 9-oxo-15β,18ξ-dihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid.
21. 9-oxo-15β,19ξ-dihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid.
22. 9-oxo-11α,15α,18ξ-trihydroxy-prost-13(t)-enoic acid.
23. 9-oxo-11α,15α,19ξ-trihydroxy-prost-13(t)-enoic acid.
24. 9-oxo-11α,15α,18ξ-trihydroxyprosta-5(c),13(t)-dienoic acid.
25. 9-oxo-11α,15α,19ξ-trihydroxy-prosta-5(c),13(t)-dienoic acid.
26. 9α,11α,15α,18ξ-tetrahydroxy-prosta-5(c),13(t)-dienoic acid.
27. 9α,11α,15α,19ξ-tetrahydroxy-prosta-5(c),13(t)-dienoic acid.
28. 9α,15α,18ξ-trihydroxy-20-ethyl-prost-13(t)-enoic acid.
29. 9α,15α,19ξ-trihydroxy-20-ethyl-prost-13(t)-enoic acid.
30. 9α,15α,18ξ-trihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid.
31. 9α,15α,19ξ-trihydroxy-15β-methyl-20-ethyl-prost-13(t)-enoic acid.
32. 9α,15β,18ξ-trihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid.
33. 9α,15β,19ξ-trihydroxy-15α-methyl-20-ethyl-prost-13(t)-enoic acid.
34. 9α,15β,20-trihydroxy-prost-13(t)-enoic acid.
35. 9β,15β,20-trihydroxy-prost-13(t)-enoic acid.
36. 9-oxo-15α,20-dihydroxy-prosta-5(c),8(12),13(t)-trienoic acid.
37. 9-oxo-15α,20-dihydroxy-prosta-5(c),13(t)-dienoic acid.
38. 9-oxo-15α,20ξ-dihydroxy-15β-methyl-20ξ-ethyl-prost-13(t)-enoic acid.
39. 9-oxo-15β,20ξ-dihydroxy-15α-methyl-20ξ-ethyl-prost-13(t)-enoic acid.
40. 9α,15α,20ξ-trihydroxy-20ξ-ethyl-prost-13(t)-enoic acid.
41. 9α,15α,20ξ-trihydroxy-15β-methyl-20ξ-ethyl-prost-13(t)-enoic acid.

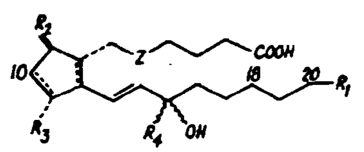
42. 9α,15β,20ξ-trihydroxy-15α-methyl-20ξ-ethyl-prost-13(t)-enoic acid.
43. 9-oxo-15α,20-dihydroxy-prost-13(t)-enoic acid.
44. 9-oxo-15β,20-dihydroxy-prost-13(t)-enoic acid.
45. 9β,15α,20-trihydroxy-prost-13(t)-enoic acid.
46. methyl 9-oxo-11α,15α,18ξ-trihydroxy-prost-13(t)-enoate.
47. 9-oxo-11α,15α,19ξ-trihydroxy-prost-13(t)-enoic acid triethanolamine salt.
48. Process for the preparation of 18ξ-, 19ξ- and 20ξ-hydroxy-prostaglandin derivatives of the general formula IA,


IA

wherein the dotted line in the position 8—12 indicates the optional presence of a double bond, the wavy lines in position 15 indicate that the hydroxyl group and the group R_4 are in either the α- or β-configuration and Z represents a $—CH_2CH_2—$ or a *cis* —CH=CH— group, and wherein R represents on of the groups:

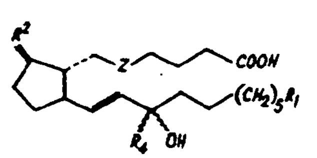


(wherein the wavy lines indicate that the hydroxyl groups are in either the α- or β-configuration and R_1 represents a hydrogen atom, a methyl or ethyl group), R_2 represents either an oxygen atom or a β- or α-hydrogen atom and an α- or β-hydroxyl group, R_3 represents a hydrogen atom or a hydroxyl group and R_4 represents a hydrogen atom or a methyl group with the proviso that (1) when there is a double bond in the 8—12 position, R_3 does not represent a hydroxyl group and (2) when there is a double bond in the 8—12 position, R_2 does not represent a β- or α-hydrogen and an α- or β-hydroxyl group; which comprises subjecting a compound of the general formula II,


II

wherein the dotted line in the position 10—11 indicates the optional presence of a double bond in which case the 8—12 position is saturated and R_3 represents hydrogen, and the other symbols are as defined above, to the hydroxylating activity of (i) microorganisms (or enzymes thereof) of the Division of *Eumycota* or, (ii) when it is desired to prepare an 18- or 19-hydroxy prostaglandin derivative, microorganisms (or enzymes thereof) of the Family of *Streptomycetaceae* and, if desired, converting the resulting hydroxy-prostaglandin derivative of formula I into a pharmaceutically acceptable salt or alkyl ester thereof, with the proviso that when the microorganism is *Cunninghamella blakesleena* (ATCC 9245), the compound of formula II is not 15(*S*)-hydroxy-9-oxo-prosta-5(C),10(t),13(t)-trienoic acid (PGA_2).

49. Process according to Claim 48, wherein the compound which is subjected to hydroxylating activity is a compound of the general formula III,


III

wherein Z, R_1, R_2 and R_4 are as defined in Claim 48.

50. Process according to claim 48 or 49, wherein the compound which is subjected to hydroxylating activity is

9-oxo-15α-hydroxy-prosta-5(c),10,13(t)-trienoic acid,
9-oxo-15α-hydroxy-prosta-5(c),8(12),13(t)-trienoic acid,
9-oxo-11α,15α-dihydroxy-prost-13(t)-enoic acid,
9-oxo-11α,15α-dihydroxy-prosta-5(c),13(t)-dienoic acid,
9α,11α,15α-trihydroxy-prost-13(t)-enoic acid,
9β,11α,15α-trihydroxy-prost-13(t)-enoic acid,
9α,11α,15α-trihydroxy-prosta-5(c),13(t)-dienoic acid or
9β,11α,15α-trihydroxy-prosta-5(c),13(t)-dienoic acid.

51. A process according to any one of claims 48—50, wherein the microorganism (or enzyme thereof) is of the Division of *Eumycota* or *Streptomycetaceae* and an 18- or 19-hydroxy-prostaglandin is obtained.

52. A process according to claim 51, wherein the microorganisms are of the Orders *Oomycetes, Coelomycetes, Hyphomycetes, Gasteromycetes, Hymenomycetes, Plectomycetes, Pyrenomycetes, Loculoascomycetes* or *Zygomycetes*.

53. A process according to claim 51, wherein the microorganisms are of the genus *Streptomycetes*.

54. A process according to any one of claims 48—50, wherein the microorganism (or enzyme thereof) is of the Division of *Eumycota* and a 20-hydroxy prostaglandin is obtained.

55. A process according to claim 54, wherein the microorganisms are of the Orders *Oomycetes, Coelomycetes, Hyphomycetes, Gasteromycetes, Hymenomycetes, Plectomycetes, Pyrenomycetes, Loculoascomycetes* or *Zygomycetes*.

56. A process according to claim 48, substantially as hereinbefore described with reference to any one of the Examples.

57. A hydroxy prostaglandin derivative obtained by a process according to any one of claims 48—56.

58. An 18- or 19-hydroxy prostaglandin derivative obtained by a process according to claim 51.

59. A 20-hydroxy prostaglandin derivative obtained by a process according to claim 54.

60. A pharmaceutical composition comprising, as active ingredient, a hydroxy prostaglandin derivative according to any one of claims 1—47 or 57—59, together with a pharmaceutically acceptable carrier.

61. A composition according to claim 60, wherein the active ingredient is in accordance with claim 2 or 58.

62. A composition according to claim 60, wherein the active ingredient is in accordance with claim 3 or 59.

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Reference has been directed in pursuance of section 9, subsection (1), of the Patents Act 1949, to patents No's. 1,314,292, 1,314,291, 1,163,762, 1,120,243, 1,097,533, 1,097,157 and 1,040,544.

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Fig. 1

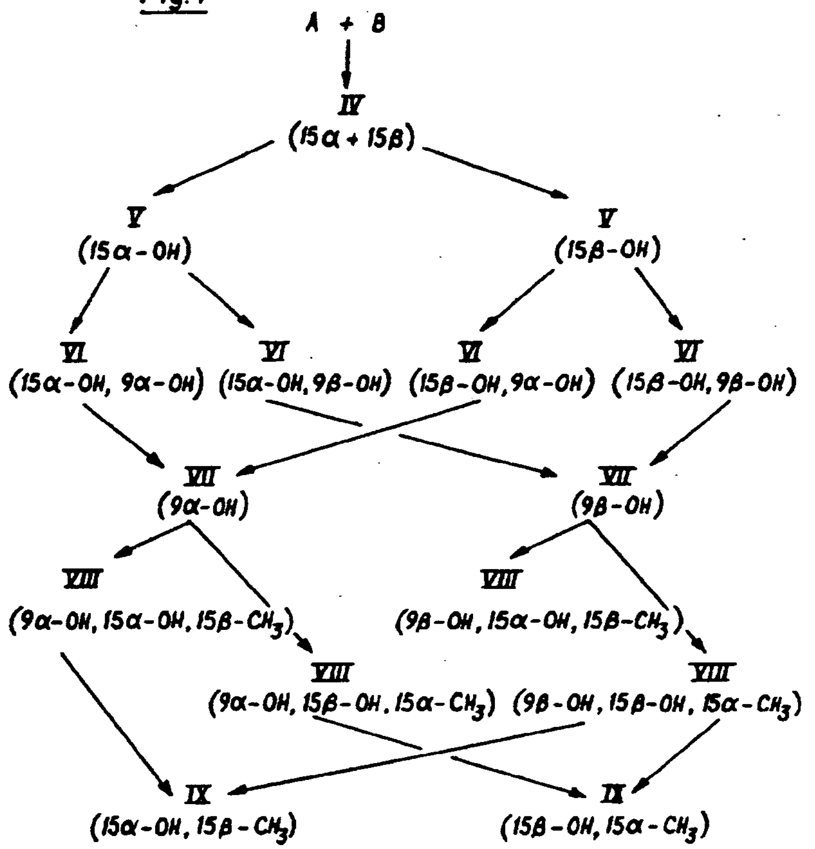
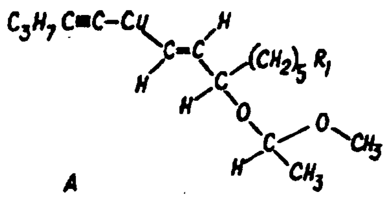
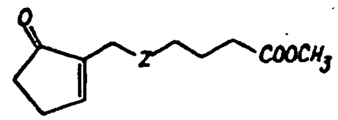

A + B
IV
(15α + 15β)
V
(15α-OH)
V
(15β-OH)
VI
(15α-OH, 9α-OH)
VI
(15α-OH, 9β-OH)
VI
(15β-OH, 9α-OH)
VI
(15β-OH, 9β-OH)
VII
(9α-OH)
VII
(9β-OH)
VIII
(9α-OH, 15α-OH, 15β-CH3)
VIII
(9β-OH, 15α-OH, 15β-CH3)
VIII
(9α-OH, 15β-OH, 15α-CH3)
VIII
(9β-OH, 15β-OH, 15α-CH3)
IX
(15α-OH, 15β-CH3)
IX
(15β-OH, 15α-CH3)

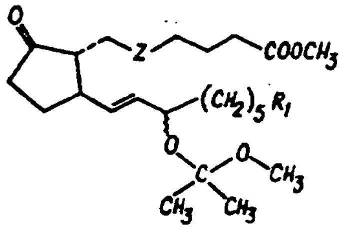
Fig. 2



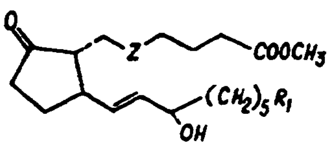
A



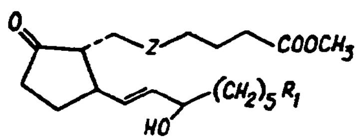
B



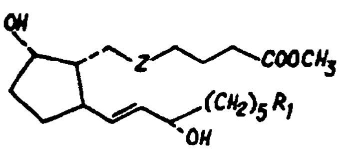
IV



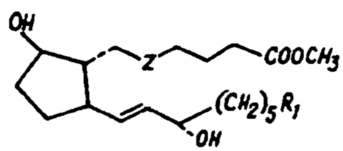
V
(15α-OH)



V
(15β-OH)

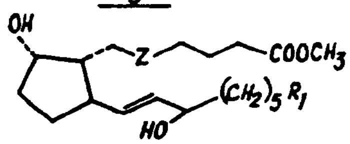


VI
(15α-OH, 9α-OH)

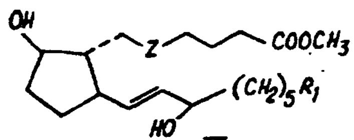


VI
(15α-OH, 9β-OH)

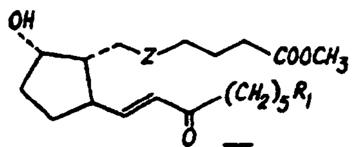
Fig. 2 - cont'd



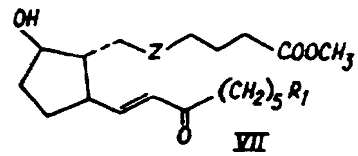
VI
(15β-OH, 9α-OH)



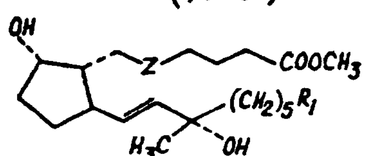
VI
(15β-OH, 9β-OH)



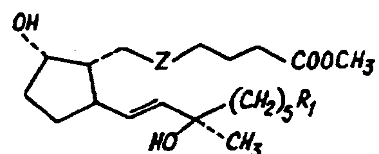
VII
(9α-OH)



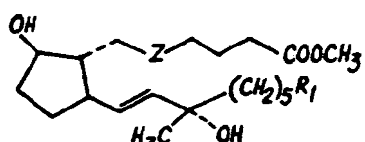
VII
(9β-OH)



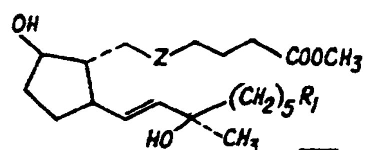
VIII
(9α-OH, 15α-OH, 15β-CH_3)



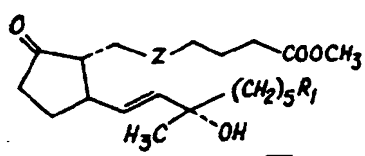
VIII
(9α-OH, 15β-OH, 15α-CH_3)



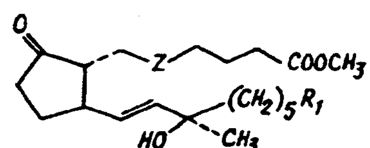
VIII
(9β-OH, 15α-OH, 15β-CH_3)



VIII
(9β-OH, 15β-OH, 15α-CH_3)

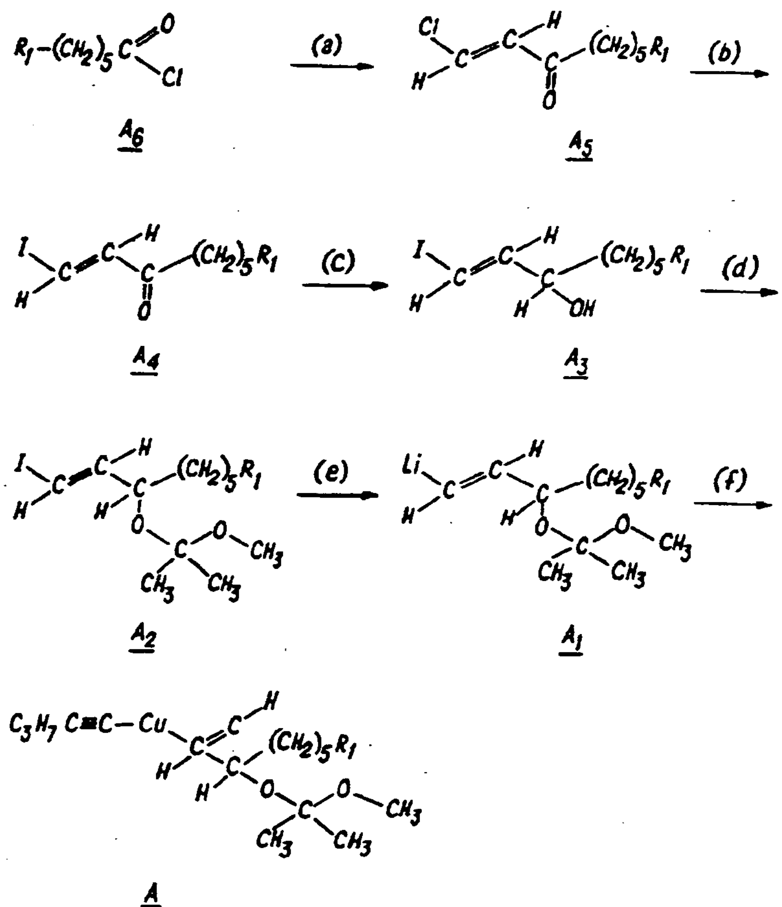


IX
(15α-OH, 15β-CH_3)



IX
(15β-OH, 15α-CH_3)

Fig. 3



(R_1=H, CH_3 or C_2H_5)

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